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INSECT–PLANT BIOLOGY

Louis M. Schoonhoven | Joop J.A. van Loon | Marcel Dicke

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Second Edition

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This book is dedicated to Vincent G. Dethier, Tibor Jermy, John S. Kennedy, and Jan de Wilde, whose pioneering contributions paved the way of modern research in the biology of insect–plant interactions

Foreword to the second edition

It is widely known that plants and insects make up approximately half of all known species of multicellular organisms. They have been evolving together for over one hundred million years, with varying levels of interaction leading to the selection of characters that are studied today in every branch of biology, from biochemistry and genetics to behaviour and ecology.

The interactions between insects and plants may be mutualistic, as with some specialized flower-pollinator pairs, and the overall importance of insects as pollinators gave rise to the earliest detailed studies of plant-insect relationships two hundred years ago. Antagonistic interactions, in particular between crops and herbivorous insects, have provided the inspiration for many studies in the past hundred years, leading to greater understanding of plant chemistry, insect physiology and behaviour and ecology. The ability to establish molecular phylogenies has led to renewed interest in the degree of co-evolution of plants with insects. In these studies, complexities revealed themselves in the form of multiple interactions—with microorganisms, fungi, predators and parasitoids of insects—all impacting how interactions between plants and insects develop, vary, and evolve. By contrast, many examples of herbivores being inconsequential in the lives of plants have also been found.

Today, the field of plant-insect interactions encompasses the whole gamut of relationships, across the complete range of biological disciplines. We think, not just of an insect herbivore choosing to eat an undefended plant, but of the many historical factors that influence the choice made, the phenotypic plasticity of behaviour, the different trophic levels playing a part in the response, the sensory and neural determinants of the choice, the consequences of it for the herbivore, the plant genes activated in their turn and the potential selection pressures elicited in either or both directions. We know that ecological as well as physiological factors influence diet breadth. We know that an insect population restricted to a plant species may become genetically isolated in the first step of a speciation event. We recognize that while insect evolution follows plant evolution the reciprocity of close co-evolution is not necessarily present.

This book amply demonstrates the breadth of the field, with the three authors bringing their combined expertise and experience to all aspects of Insect–Plant Biology. From the vast literature on the topic the authors have selected examples of experiment and theory to produce a book that is a comprehensive guide for students and researchers alike. From an overview of the patterns found in nature, they move logically to plant structure and chemistry, host finding and host choice, including variation and insect physiology. The later chapters include coverage of ecology and evolution, insect and flower interactions, and last, to applications of knowledge in insect-plant interactions.

Since 1998, when the first edition of this book was published, advances have been made particularly in plant biochemistry and evolution. Molecular techniques have elucidated details in these and other areas, and the understanding of insect-plant biology has broadened. With the inclusion of Marcel Dicke among the authors, some of the newer work as well as the topic of co-evolution get a somewhat different treatment, and throughout the book, there are additions and updates. For anyone with an interest in any aspect of plant and insect interactions, this text will be a firm and reliable resource.

Elizabeth A. Bernays
University of Arizona
Tucson
May 2005

Preface to the second edition

Apart from offering an update motivated by the rapidly expanding literature of the past seven years, this second edition differs in two respects from the previous one. First, Dr. Tibor Jermy, one of the founders of the field of insect-plant relations and co-author of the first edition, bears no responsibility for the present book. Yet his extensive knowledge and views on the subject can be traced back in the present edition. It is with pleasure that we dedicate this volume to Tibor Jermy, to express our respect for his deep insights and long-lasting contributions to the development of insect-plant biology.

The second difference with the first edition relates to recent changes in the scientific approach of the multifaceted way in which insects and plants interact with each other and with their environment. New insights into, among others, the molecular biology of chemoreception and induced plant defences and their effects on higher trophic levels allow a more comprehensive approach of the theme of this book than could be envisaged only a few years ago. Studying insect-plant interactions at the molecular level adds an exciting dimension to our understanding.

We are indebted to several colleagues, who have read and commented on chapters, including Tibor Jermy, Peter de Jong, Erich Städler, and Freddy Tjallingii. We thank them for their encouragement and help. Hans Smid produced some marvellous new photographs and several other colleagues have allowed us to reproduce some of their finest micrographs. M.D. gratefully acknowledges the NERC Centre for Population Biology of Imperial College at Silwood Park (UK) and its director Charles Godfray, as well as Maarten and Elly Koornneef (Cologne, Germany) for providing a hospitable and inspiring environment when working on this second edition.

We are especially grateful to everyone at the Production Department of Oxford University Press for their stimulation and help with turning our text into what we hope will be a useful and stimulating book.

Wageningen, Summer 2005 *L.M. S.*
J.J.A. v. L.
M. D.

Preface to the first edition

Green plants cover most of the terra firma on planet Earth. Insects are dominant among plant consumers. The interactions between plants trying to avoid consumption, and insects trying to optimize food exploitation, are the subject of this book. It is a rich subject: the primary literature has grown during the past 25 years at an exponential rate. It is also an intellectually challenging subject since, in spite of the wealth of facts, the principles underlying insect–plant interactions are still largely unknown. This book aims to categorize the multitude of facts derived from studies in natural surroundings as well as agricultural environments, and attempts to indicate emerging lines of understanding. Hopefully it will serve as an introduction to students of this area of biology and will highlight to general biologists the complexity of interactions between organisms.

The need for increased agricultural production, together with the necessity to reduce the use of insecticides, forces agricultural entomologists to study how plants in nature have survived insect attack over the aeons, and whether these defence systems can be adopted in agricultural settings. Therefore this book may also be helpful to applied entomologists, who are in search of new ways to protect our daily food production.

The information abounding in the recent literature is too extensive to attempt any complete review. Therefore we have selected studies which were especially appealing to us. In this process we must have missed other equally (or more) important reports and opinions, for which we apologize. In addition to trying to offer an objective representation of facts and thoughts as found in the existing literature we have unavoidably, but also deliberately, given some personal views as well.

We wish to dedicate this book to the memory of three great men who have deeply influenced our thoughts on this subject and who can be considered as founding fathers of the field: Jan de Wilde, Vincent G. Dethier, and John S. Kennedy. Without their foresight, their stimulating enthusiasm, and their perceptiveness of basic mechanisms operative in nature the field of insect–plant relationships would not have reached its present prominence.

Many people have provided generous assistance in a variety of ways—stimulating discussions, frank criticism, the provision of material for illustrations, and permission to use published diagrams and information. We should especially like to mention those who have read parts of the manuscript and made useful suggestions for improvement: T.A. van Beek, J. Beetsma, M. Dicke, P. Harrewijn, M. van Helden, J.C. van Lenteren, S.B.J. Menken, L. Messchendorp, C. Mollema, P. Roessingh, E. Städler, Á. Szentesi, W.F. Tjallingii, and H.H.J. Velthuis. Last but not least our thanks also go to the staff of Chapman & Hall for seeing the book efficiently through production.

Wageningen, Budapest *L.M. S.*
Autumn 1996 *T. J.*
J.J.A. v. L.

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CHAPTER 1

Introduction

Almost a century and a half ago W. Kirby and W. Spence described in their legendary book on entomology[3] the flight of a large white butterfly in pursuit of a suitable plant to lay eggs on: 'she is in search of some plant of the cabbage tribe. Led by an instinct far more unerring than the practised eye of the botanist, she recognizes the desired plant the moment she approaches it; and upon this she places her precious burden.' The authors then pose one of the basic questions, which has occupied scholars of insect–plant relationships till the present day: 'But how is she to distinguish the cabbage plant from the surrounding vegetables?' The answer given—'She is taught of God!'[3]—shows that the precise relationship between herbivorous insects and their host plants has for a long time defied causal analysis. Scientific inquiry into the mechanisms of host-plant selection by herbivorous insects started around 1900,[2,9] but for a long time roused curiosity among only a few biologists. Roughly half a century ago zoologists began the causal analysis of insect behaviour such as host-plant discrimination, and gradually some insight has been gained into the underlying mechanisms.

1.1 Increased attention: why?

There are several reasons why insect–plant interactions are receiving increasing attention from biologists as well as agronomists. It is now recognized that, from the perspective of fundamental knowledge of the earth's biosphere, the relationships between insects and plants are of crucial importance. First there is the quantitative factor: the Plant Kingdom and the class of insects represent two very extensive taxa of living organisms, both in abundance of species and in amount of biomass. Green plants form by far the most voluminous compartment of living matter (Fig. 1.1), whereas insects are the leaders in number of species.

As ecologist Robert May[6] puts it: 'To a rough approximation, and setting aside vertebrate chauvinism, it can be said that essentially all organisms are insects.' Certainly not only their variety but also their total volume is colossal, in spite of their small body sizes. For instance, the biomass of all insects in temperate terrestrial ecosystems outweighs that of the total land vertebrate population by about 10 to 1 (Fig. 1.2).[7]

1.2 Relationships between insects and plants

The two empires, herbivorous insects and plants, are united by intricate relationships. Animal life, including that of insects, cannot exist in the absence of green plants, which serve as the primary source of energy-rich compounds for heterotrophic organisms. On the other hand, long-standing exposure to animals has supposedly been a major cause in developing great diversity in the plant world. Insects, with their overwhelming variation in form and life history, may have been one of the forces in shaping the plant world.[5] Such a role has been postulated by Ehrlich and Raven,[1] who in a seminal paper attributed the plant–herbivore interface as the major zone of interaction for generating the present diversity of terrestrial life forms.

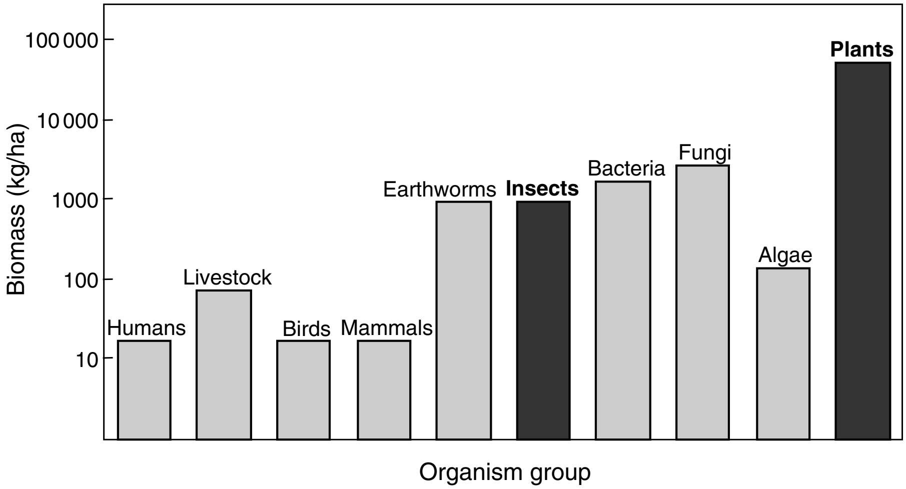


Figure 1.1 The average biomass of humans, their livestock, and the estimated biomass of natural biota of some other major groups of organisms per hectare in the USA. Insects include also non-insect arthropods. Note logarithmic scale. (Data from Pimentel and Andow, 1984.)[7]

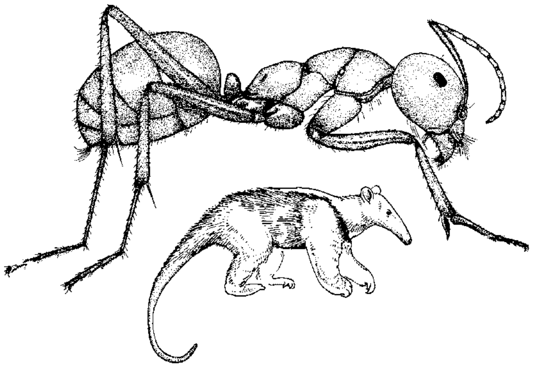


Figure 1.2 In this drawing the ant represents the biomass of all ant populations and the ant-eater the biomass of all land vertebrates in the Brazilian Amazon. The ants alone outweigh the vertebrates by about four to one. If all other social and non-social insects were included, this ratio would be nine to one. (Drawings by R.J. Kohout and E. Wright.)

The terrestrial flowering plants are the *sine qua non* of the insect tribe, for it is among the insects that feed upon these that herbivory reaches its highest degree of specialization. Such species present a series of complex relationships that are more easily understood if we first consider separately several of their peculiarities. Probably no other interactions between two groups of organisms comparable in type and extent can be found elsewhere in the living world, thus rendering insect–plant interactions a unique and scientifically very fruitful area of biological research.

1.3 Relevance for agriculture

Obviously insect–plant interactions are also of crucial importance from an applied point of view. Insects remain, and may even have increased significance as, the chief pests of crops and stored products, despite expensive and environmentally hazardous control measures (Fig. 1.3).

There is an irrefutable need to understand better the factors governing the relationships between insects and plants, as this may help to unravel the causes of insect pest development. Such knowledge is fundamental when attempting to create biologically safe control strategies intended to prevent insect pest outbreaks. The study of insect–plant relationships therefore constitutes, as Lipke and Fraenkel[4] aptly put it, 'the very heart of agricultural entomology.'

1.4 Insect–plant research involves many biological subdisciplines

Insect–plant interactions include problems at different levels of biological analysis. Questions such as

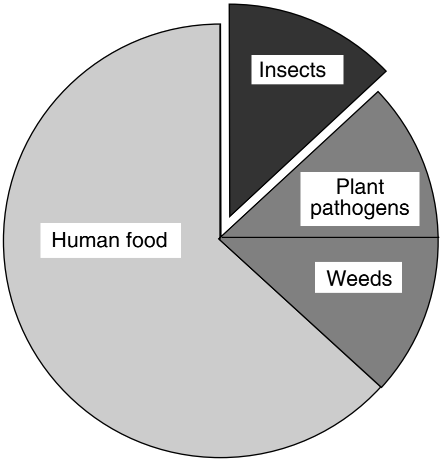


Figure 1.3 Estimated potential crop losses during preharvest to insects (13%), diseases (12%), and weeds (12%) in the USA. (Data from Pimentel, 1997.)[8]

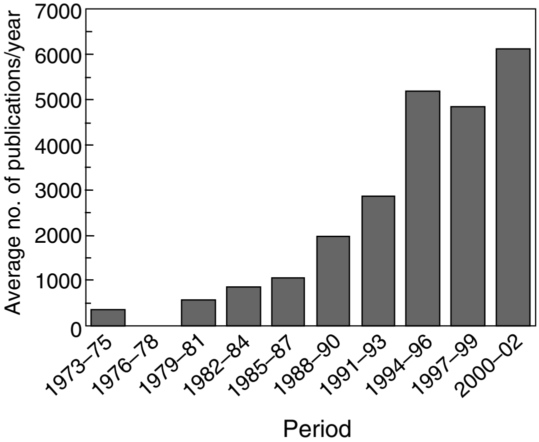


Figure 1.4 Primary references (from 1973 to July 2002) drawn from the Commonwealth Agricultural Bureau (CAB) database. The bars represent the average numbers per year of all publications fitting the search statement (insect or mite) AND (plant or host plant) AND (journal article). All descriptor fields used.

'Why do cabbage worms devour cabbage leaves, but refuse to eat potato plants?' lie at the level of the organism, whereas the question 'Why are some forests more prone to insect outbreaks than others?' requires an ecological approach. The focus in this book is upon the mechanistic analysis at the level of the organism, including a physiological and molecular biological approach. Ecological aspects, however, have not been neglected, because insights derived from studies at the organismal level are often useful elements in ecological models. Another reason for including a discussion of ecological aspects is that the function of many behavioural or physiological traits becomes obvious only when put in an ecological perspective.

As in other biological subdisciplines, students of insect–plant interactions may be interested in proximate puzzles (how?) or in ultimate factors (why?). Questions like 'How does an insect recognize its host plant?' and 'How does a herbivore avoid being poisoned by toxic compounds in its food plant?' belong to the first category. Questions like 'Why do desert plants contain more terpenoids than species occurring in pastures?' and 'To which extent have insects stimulated the evolution of flowering plants?' relate to ultimate causes. Physiologists and molecular biologists are concerned mainly with proximate factors, whereas students of evolution concentrate on finding ultimate causes. Both approaches in fact complement each other and will therefore be employed in this book, but in many instances without explicitly referring to either type.

The topic of insect–plant interactions is too extensive to be covered comprehensively in a book of this size. The rapidly growing interest in this field is evidenced by the real flood of scientific papers (Fig. 1.4), including many extensive reviews and several books (Appendix A), published during the past two or three decades. The amount of information becoming available cannot be collected, let alone absorbed by a single individual. The following text therefore attempts to derive general principles, illustrated with a limited number of specific examples. As the species-to-species variation in behavioural responses and physiological adaptations is huge, the reader interested in a specific insect–plant relationship is referred to reviews or the primary literature.

1.5 References

1. Ehrlich, P.R. and Raven, P.H. (1964). Butterflies and plants: a study in coevolution. *Evolution*, **18**, 586–608.
2. Errera, L. (1886). Un ordre de recherche trop négligé. L'efficacité des structures défensives des plantes.

Comptes Rendus des Séances de la Société Royale de Botanique Belgique, **25**, 80–99.

3. Kirby, W. and Spence, W. (1863). *An introduction to entomology* (7th edn). Longman, Green, Longman, Roberts & Green, London.
4. Lipke, H. and Fraenkel, G.S. (1956). Insect nutrition. *Annual Review of Entomology*, **1**, 17–44.
5. Marquis, R.J. (2004). Herbivores rule. *Science*, **305**, 619–21.
6. May, R.M. (1988). How many species are there on earth? *Science*, **241**, 1441–9.
7. Pimentel, D. and Andow, D.A. (1984). Pest management and pesticide impacts. *Insect Science and its Application*, **5**, 141–9.
8. Pimentel, D. (ed.) (1997). *Techniques for reducing pesticides: environmental and economic benefits*. Wiley, Chichester.
9. Verschaffelt, E. (1910). The cause determining the selection of food in some herbivorous insects. *Proceedings Royal Academy, Amsterdam*, **13**, 536–42. (Reprinted in *Proceedings Koninklijke Nederlandse Akademie van Wetenschappen*, **100**, 362–68, 1997.)

CHAPTER 2

Herbivorous insects: something for everyone

Insects have the most species of any class of organisms on earth and green plants make up the greatest part in all biomass on land. Nearly half of all existing insect species feed on living plants. Thus, more than 400 000 herbivorous (synonymous with phytophagous) insect species live on roughly 300 000 vascular plant species (Fig. 2.1).

According to some recent estimates, the total number of insect species is considerably larger than was previously thought and may range from 4 to 10 million.[82,85] If this reflects reality, the number of vegetarian species probably needs to be adjusted proportionally. The relativity of estimations of species numbers is, however, strikingly demonstrated by publications that diagnose species homogeneity by DNA sequencing. The application of such protocol to, for instance, the neotropical butterfly *Astraptes fulgerator* revealed that this species forms a mimetic assemblage of at least 10 different species, with their adults showing little phenotypic diversity, but their larvae characterized by mostly different food plants.[53]

Herbivory does not occur to the same extent in all insect groups. The members of some orders of

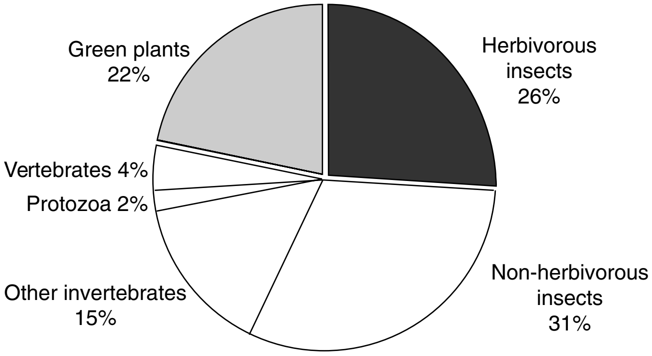


Figure 2.1 The proportions of plant and animal species in major taxa, excluding fungi, algae, and microbes. (From Strong *et al.*, 1984.)[113]

insects are almost exclusively herbivorous, whereas in other orders herbivory occurs less frequently or is even absent. Conspicuous among the herbivores are the Lepidoptera (butterflies and moths), Hemiptera (bugs, leaf-hoppers, aphids, etc.), Orthoptera (grasshoppers and locusts), and some small orders such as the Thysanoptera (thrips) and Phasmida (walking sticks). A large part of the vast horde of herbivorous insects belongs to the speciose orders Coleoptera, Hymenoptera, and Diptera, all three of which also include numerous species with predatory and parasitic habits (Table 2.1).

Table 2.1 Numbers of herbivorous species in different insect orders (data from various sources)

Insect order	Total no. of species	Herbivorous species	
		No.	%
Coleoptera	349 000	122 000	35
Lepidoptera	119 000	119 000	100
Diptera	119 000	35 700	30
Hymenoptera	95 000	10 500	11
Hemiptera	59 000	53 000	90
Orthoptera	20 000	19 900	100
Thysanoptera	5 000	4 500	90
Phasmida	2 000	2 000	100

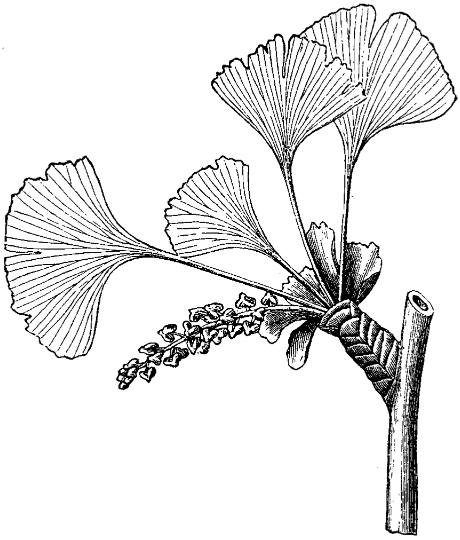


Figure 2.2 *Ginkgo biloba*. Shoot with young leaves and male inflorescence.

Given the innumerable plant-infesting insect species it is not surprising that all terrestrial tracheophytes (vascular plants) harbour some members of the herbivore tribe. Although at some time it was assumed that evolutionarily ancient plants, such as the maidenhair tree (*Ginkgo biloba*), a 'living fossil' (Fig. 2.2), and ferns were devoid of insect consumers, it is now known that this tree,[125] as well as other relicts of a glorious past, including ferns,[54,93,94] lycopods,[61,104] mosses,[66,108,110] lichens,[66,78] and mushrooms,[22] serve as food to at least some insect species.

2.1 Host-plant specialization

One of the most striking features of insect–plant relationships is the high degree of food specialization among herbivorous insects. This phenomenon forms the heart of these relationships, and all discussions in the following chapters are pervaded with this notion. It is therefore useful to consider the degree of dietary specialization or generalization shown by herbivores. Insects that in nature occur on only one or a few closely related plant species are called *monophagous*. Many lepidopterous larvae, hemipterans, and coleopterans fit into this category. *Oligophagous* insects, such as the cabbage white butterfly (*Pieris brassicae*) and the Colorado potato beetle (*Leptinotarsa decemlineata*), feed on a number of plant species, all belonging to the same plant family, the Brassicaceae and the Solanaceae, respectively. *Polyphagous* insect species seem to exercise little choice and accept many plants belonging to different plant families. The green peach aphid (*Myzus persicae*), for instance, has been recorded to feed during summertime on members of more than 50 plant families. Its winter host plant, however, is the peach tree (*Prunus persica*) or closely related rosaceous plants.

This classification into three categories, however, is fairly arbitrary, because precise definitions of monophagy and oligophagy are difficult to sustain. The first problem is that there is a completely graded spectrum between species that will eat only a single kind of plant and those that regularly consume many very diverse plants. Second, individuals of the same insect species may show different host-plant preferences in different areas of its distribution, and even individuals belonging to the same population may be much more restricted in their choices than the population as a whole.[13,57,87] In view of these observations it is often more convenient to distinguish only *specialists* (monophagous and oligophagous species together) from *generalists* (polyphagous species).

Host-plant specialization is the rule rather than the exception. It has been calculated that less than 10% of herbivorous species feed on plants in more than three different plant families.[9] Monophagy, the other extreme, is a common feature, and in certain insect groups it is even the dominant habit. An inventory of about 5000 British herbivorous insect species shows that more than 80% of them should be regarded as specialists. Different insect groups, however, may show quite different degrees of specialization. Of the 25 British orthopteran species, 51% are polyphagous whereas 41% are restricted feeders on grasses and sedges. Conversely, 76% of all British aphids are strictly monophagous, 18% are oligophagous, and only 6% are polyphagous. Monophagy is also a common habit among leaf-miners and leaf-hoppers (Fig. 2.3). As a generalization it may be stated that most orders of herbivorous insects are dominated by specialists, whereas many, if not most, grasshopper species regularly eat from more than one plant family.[16]

In discussions on host-plant specialization and its terminology[64] it has been argued that some oligophagous or even polyphagous insects should more appropriately be considered as monophagous when their host-plant selection is based upon a specific type of plant chemical. Larvae of the cabbage white butterfly, which are restricted to cruciferous plants, are occasionally also found on *Tropaeolum* (nasturtium) or *Reseda* species. Both plants belong to different families but, in common with the normal host plants of this insect, they contain glucosinolates, chemicals that typically occur in the Brassicaceae. One could say that the cabbage white butterfly is monophagous on glucosinolate-containing plants, but usage of the term in this narrow sense ignores the fact that additional plant characteristics usually play a role in host-plant selection as well. The same reasoning has been put forward to characterize the polyphagous larva of the brown-tail (*Euproctis chrysorrhoea*) as a specialist, because it feeds, like some other caterpillar species,[44] predominantly on tree species with tannins in their leaves (Fig. 2.4). For practical as well as reasons of principle we prefer, however, to relate the classification of host specialization to the range of an insect's natural host plants.

The breadth of the host-plant range shown by a particular insect species is probably one of its major biological characteristics, and is constrained by several morphological, physiological, and ecological factors. In order to uncover these constraints it may be helpful to look for correlations between diet

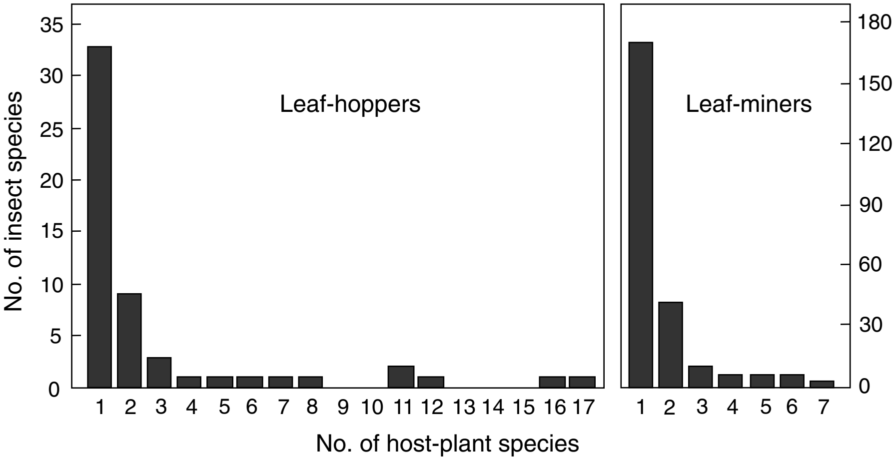


Figure 2.3 Most leaf-hoppers and leaf-miners on British trees are strict monophages. (From Crawley, 1985.)[25]

breadth and plant or herbivore characteristics. Several interesting relationships have been found. For instance, insects living on herbaceous plants often show a higher degree of host specialization than insects on shrubs and trees (Table 2.2).[14,41] This may be explained by the fact that herbaceous plant species show a greater diversity, for example in life cycle and chemical composition, than woody plants. These features mean that specialist insects adapted to these variables are in a better position than generalists to exploit such food plants.

A second noteworthy aspect of host-plant specialization is the relationship between the breadth of an insect's diet and its body size: smaller species are generally more specialized than larger species (Table 2.3).[70,80] Perhaps larger species run a greater risk of food depletion and are therefore less choosy.

A third observation to be made here is that herbivores with narrow host-plant ranges usually show a preference for young growing leaves, whereas, overall, larvae of polyphagous species prefer mature leaves of their various host plants. Young leaves are generally more nutritious,[107] but at the same time often also contain higher levels of toxic secondary plant substances than mature leaves.[14] Specialized detoxification systems are required to compensate for this drawback.

These three trends are most probably not fortuitous correlations but reflect some biological principles. Perhaps the observed patterns are related to completely different biological properties, because the frequency of strong specializations is much higher in some insect taxa than in others (Table 2.3).

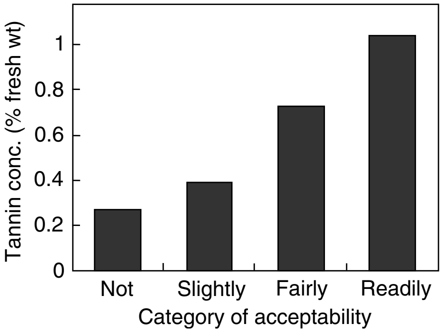


Figure 2.4 Polyphagous larvae of the brown-tail *Euproctis chrysorrhoea* favour plants with high tannin levels. Sixty-one plant species were categorized into four acceptability classes, which appear to correlate with average tannin contents. (Data from Grevillius, 1905.)[46]

Table 2.2 Host plant specialization of Lepidoptera on herbaceous and woody plant species (data from Futuyma 1976)[41]

	No. of species	% specialists
Moths and butterflies in Great Britain		
On herbaceous plants	143	69
On woody plants	229	54
Butterflies in North America		
On herbaceous plants	110	88
On woody plants	53	68

Table 2.3 Percentage of insect species within taxonomic groups that feed on plants within a single plant genus, or within a single plant family, or on more than one family of plants. (modified from Mattson *et al.*, 1988)[76]

Insect group	No. of species	% of species feeding on		
		One plant genus only	One plant family only	More than one plant family
Psyllidae (Diptera)	78	94	3	0
Aphididae (Homoptera)	445	91	7	2
Scolytidae (Coleoptera)	NA	59	38	3
Diaspididae (Homoptera)	64	58	8	34
Thysanoptera	88	56	15	29
Nymphalidae (Lepidoptera)	88	56	11	33
Lycaenidae (Lepidoptera)	89	55	14	31
Pieridae (Lepidoptera)	43	33	53	14
Papilionidae (Lepidoptera)	89	25	21	54
Other Macrolepidoptera	430	17	23	60

Note that the first five groups of insects comprise small insects compared with the other groups. The correlation between size and host-plant range, however, is low. Many examples exist of closely related, similarly sized insects that show large differences in width of host-plant ranges.

NA, data not available.

Probably the evolutionary 'choice' between becoming a specialist or a generalist depends on a large number of heterogeneous factors. There is still a long way before we can understand why, for instance, lycaenid butterflies in the tropics are significantly more often generalists than specialists, in contrast to confamilial species in temperate climates,[35] and why the opposite holds for the Papilionidae.[102]

Polyphagous insect species may feed on a great diversity of plant species but certainly do not accept all green plants indiscriminately. Even notoriously catholic feeders are restricted to a few hundred plant species (Table 2.4), while plants outside this range are hardly fed upon or are totally rejected, even in the absence of any alternative food source.

Nor must it be imagined that polyphagous and oligophagous species are indiscriminate in what they choose from their acceptable host-plant range. On the contrary, some degree of preference is almost always apparent. Even archetypal polyphages such as the desert locust *Schistocerca gregaria*, which feeds on a wide range of plants belonging to many different families, exhibit pronounced preferences for particular plants, eating some species in small amounts and others in large amounts.[15] Another insect with a wide spectrum of host plants, the gypsy moth larva (*Lymantria dispar*), not only prefers certain oak species over others,[38] but exhibits still more discrimination. In a choice situation it shows a predilection for leaves grown on the sunny side of an alder tree over those collected at the north side of the same tree. In addition, although it will feed greedily on young expanding leaves, when offered a choice it clearly prefers full-grown leaves (Fig. 2.5). Other insects, too, are known to prefer certain compass sections of trees over other parts of the canopy.[111]

At first sight, generalists seem to be privileged compared with specialists because they have access to a much broader food range. However, Bernays[7] has argued that, owing to the limited neural capacity of insects, they are in a disadvantageous position. Specialists need to make decisions based on a smaller number of options than generalists, thereby increasing the efficiency of the decision-making process in the former group.

Table 2.4 Number of plant species infested by some polyphagous insect species

Insect species	No. of plant species infested	No. of plant families	Reference
Bemisia tabaci (cotton whitefly)	506	74	17
Lymantria dispar (gypsy moth)	>500	>22	68
Schistocerca gregaria (desert locust)	>400	53	8, 120
Lygus lineolaris (tarnished plantbug)	385	55	131
Popilia japonica (Japanese beetle)	>300	79	90
Liriomyza trifolii (serpentine leaf-miner)	>400	25	109

Note that these examples of extreme polyphagy belong to five different orders.

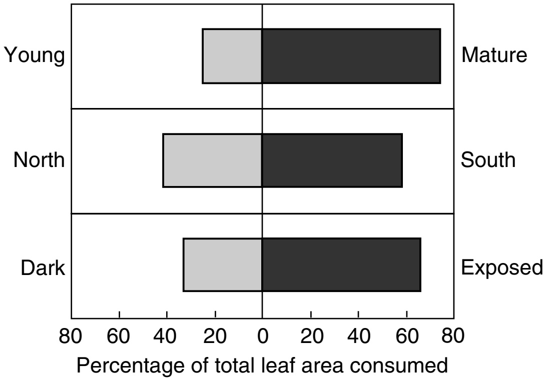


Figure 2.5 When, in a choice test, leaves of alder (*Alnus glutinosa*) were offered to gypsy moth larvae (*Lymantria dispar*), the caterpillars ate more from mature leaves than from young leaves. Likewise they preferred leaves picked from the south side of the tree over those facing north, and leaves that were exposed to normal light over leaves that were kept in the dark for 24 h. (Data from Schoonhoven, 1977.)[99]

In addition to neural (behavioural) aspects, however, many other physiological and ecological factors undoubtedly also affect the host-plant range of a herbivorous species.[59] The fact that some insect species thrive on a very broad diet, whereas others are extremely finicky in their food choice, indicates that both feeding types must have their merits and demerits.

2.2 Food-plant range and host-plant range

In many herbivorous insect species the ovipositing female selects the plant on which its offspring will feed, and the question arises whether or not host-plant choice by the ovipositing adult is identical with the food-plant range of the larval stage. Although, as would be expected, the two host ranges show a fairly close similarity, they are often not identical. This observation indicates that host selection behaviour in the ovipositing female is governed by different parts of the genome than those coding for food selection behaviour in the larva.[127] Interestingly, the diet breadth of the larvae is often wider than the range of plants accceptable as oviposition substrate to the adult female (Fig. 2.6).[45]

Obviously, natural selection will prevent the development of too great a discrepancy between the preferences of ovipositing females and their offspring. Several studies have addressed the question of whether or not the oviposition preferences of herbivorous insects fully match the performance of their offspring on these food plants in terms of survival, growth, and reproduction. In general there appears to be a good association: females preferentially oviposit on plants on which their offspring perform best.[67] This applies to female choices between different plant species,[24] as well as to choices between different plant parts (Fig. 2.7). Although, overall, the larvae of *Papilio machaon* show high survival rates on the plants that are acceptable to their mother insects, some asymmetry does exist between oviposition preference and larval performance (see Fig. 2.6).

Several cases of poor associations have been reported in the literature.[39,50,116] One explanation for such discrepancies may be the fact that most reports of a bad fit between oviposition preference and larval performance consider the correlation between mean oviposition preference and physiological

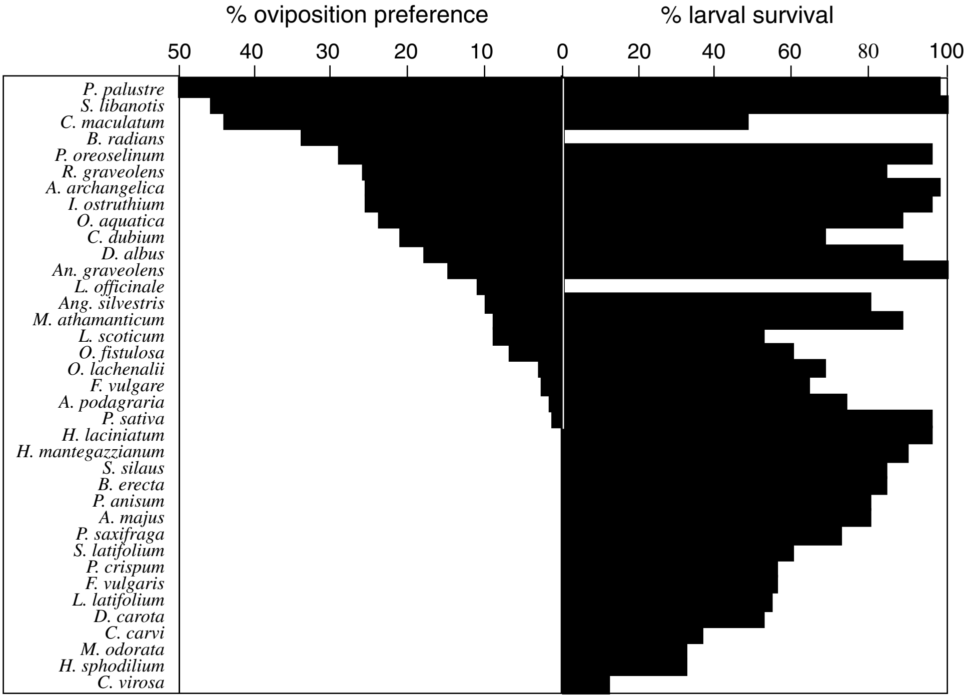


Figure 2.6 Female swallowtail butterflies (*Papilio machaon*) show a hierarchy of oviposition preferences (*left*). Most plants are suitable food plants for larvae (*right*), although females lay eggs on *Bifora radians*, which does not support larval growth. Larvae show also high survival rates on some plant species that are not selected for oviposition. (From Wiklund, 1975.)[127]

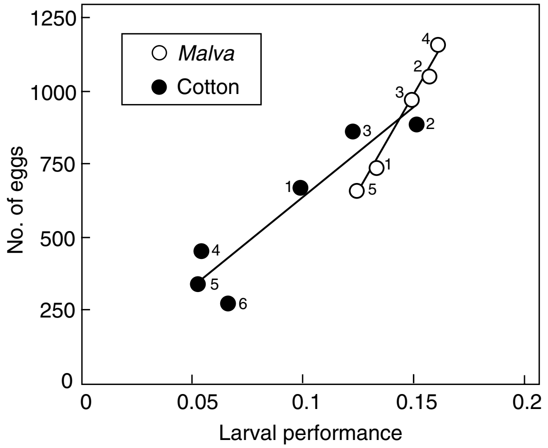


Figure 2.7 Correlation between number of eggs laid by tipworm (*Crocidosema plebejana*) females on two host plants, cotton (*Gossypium hirsutum*) and *Malva parviflora*, and larval performance (expressed as capacity for increase, r_c). Host plants were offered in no-choice situations and at different developmental stages, varying between the seedling stage (1) and senescing (*Malva*, 5) or open (cotton, 6) bolls. There is a strong correlation between the acceptability level of a plant as an oviposition substrate and its developmental stage, reflecting its nutritional adequacy for larval performance. (From Hamilton and Zalucki, 1993.)[47]

suitability averaged across many females in a herbivore population. The relevant question from the perspective of natural selection is, however, whether *individuals* select the best hosts for *their own* offspring.[60] A study of individual oviposition choices and the survival and growth of larvae did indeed show a positive correlation between both parameters.[105] Different females of the same population of the butterfly *Euphydryas editha* showed different host-plant preferences for oviposition. Larval performance of their offspring on the different host plants appeared to be correlated with maternal oviposition preference. Thus, larvae grew best on the plants that their mothers had selected.[105]

Cases of dissimilarity represent imperfect adaptations, which are perhaps due to a lack of adequate genetic variation in the alleles that determine oviposition preference or other constraints. Alternatively, a hitherto unknown factor other than larval growth and survival may drive the evolution of host-plant use,[122] or experimental procedures may be (partly) responsible for the observed imperfections.

2.3 Specialization on plant parts

2.3.1 Above-ground herbivory

Insects may consume every anatomical part of plants but, in addition to host-plant specialization, also show specialization with regard to the feeding sites they occupy on their hosts. Insects of a given species rarely thrive equally well on all parts of their host plant. Many caterpillars, beetles, and grasshoppers are leaf foragers (folivores), ingesting relatively large chunks of leaf material. Other insects show more specific needs. Thus, plant-bugs often penetrate epidermal cells and ingest cell contents, whereas aphids suck mainly from the sap flow in phloem sieve elements. Spittlebugs and cicadellinine leaf-hoppers often tap the xylem.[117] Leaf-mining insects live and feed during their larval stage between the upper and lower epidermis of a leaf-blade and devour parenchymal tissues (Fig. 2.8).

Different species may excavate different layers of the leaf parenchyma. Leaves of birch, for example, are attacked by two hymenopterous leaf miners, one of which, *Fenusa pumila*, feeds on the entire mesophyll, whereas the larvae of *Messa nana* feed only on palisade parenchyma.[28,97] Furthermore, leaf-mining species often show a predilection for particular parts of a leaf. Some tunnel near the midrib of the leaf, whereas others are usually found near the periphery of the lamina (Fig. 2.9).

Thus, different leaf parts taste different and possess different physical properties, affecting not only leaf-miners but also insects ingesting leaf pieces. Larvae of several moth species (e.g. *Catocala* spp. and *Lymantria dispar*) can discriminate between the basal, lateral, and terminal leaflets of their compound-leaved food plants, and show a dislike of basal leaflets (Fig. 2.10).[42] Plant stems may harbour stem-borers, mainly lepidopterous, dipterous, and coleopterous larvae (Fig. 2.11), and the bark of woody plants is often infested by bark beetles (Scolytidae and others).

Wood may contain the larvae of some Lepidoptera, Coleoptera, and Hymenoptera, which are adapted to this extremely unbalanced diet. Other insects are specialist feeders on flowers, fruits, or seeds, and members of several insect orders induce the formation of galls in various plant parts.[128]

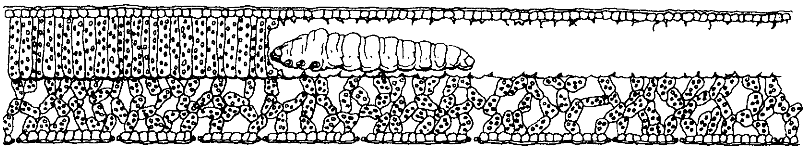


Figure 2.8 Cross-section of a leaf with a beetle larva mining in palisade parenchyma. (From *Insect Biology* by E.A. Evans. © 1984 by Addison Wesley Publishing Company. Reprinted by permission.)

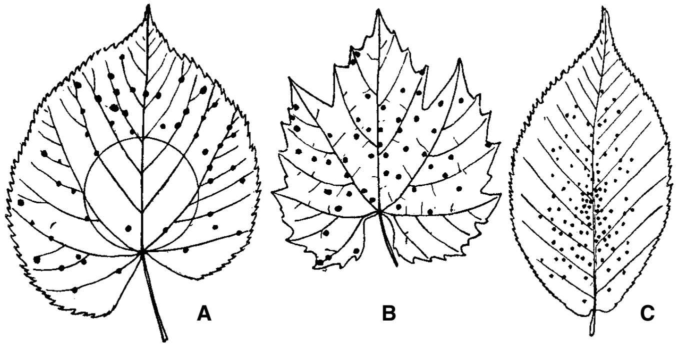


Figure 2.9 Distribution of mines of three hosts. (**A**) 50 mines of *Brachys* on lime (*Tilia* sp.). (**B**) 50 mines of *Antispila viticordifoliella* on grape (*Vitis vinifera*). (**C**) 100 mines of *Lithocolletis ostryarella* on hophornbeam (*Ostrya* sp.). (From Frost, 1942, with permission.)[40]

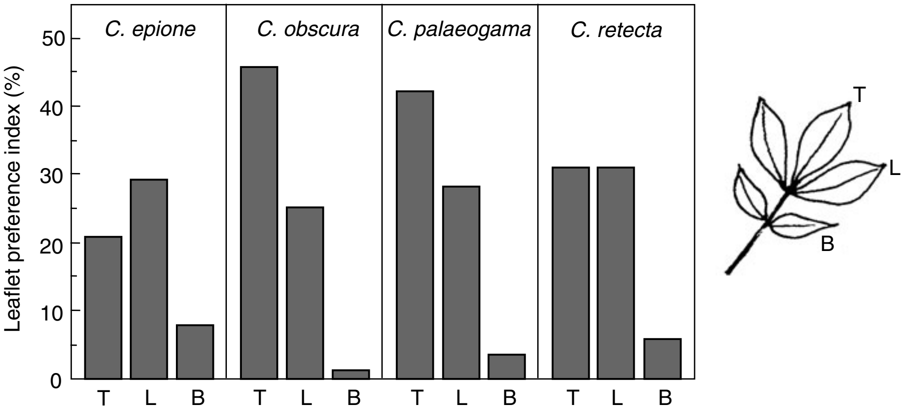


Figure 2.10 Caterpillars of four *Catocala* spp. eat less basal (B) leaf material than material from terminal (T) and lateral (L) parts of hickory (*Carya*) leaves. *Right*: compound leaf of *C. ovata*. (From Gall, 1987.)[42]

Taken together these examples show how all parts of the plant are 'shared out' and can support some insect or other.

The endless variation in adaptations to certain plant tissues is, at least to some extent, due to nutritional factors. The dietary value of different plant parts, and even of different tissues, is so different that it is not surprising to find that most small insects are specialists. The smaller the herbivore's body size, the finer the scale of heterogeneity of the plant tissues it meets. For instance, the larvae of a polyphagous pest insect *Mamestra configurata*, when feeding on the pods of rape, one of their host plants, remain smaller and show a 30% increase in mortality rate compared with conspecific larvae feeding on foliage.[11] The larvae of *Dasineura brassicae*, on the other hand, are specialized feeders on the pods of rape and survive only on these plant

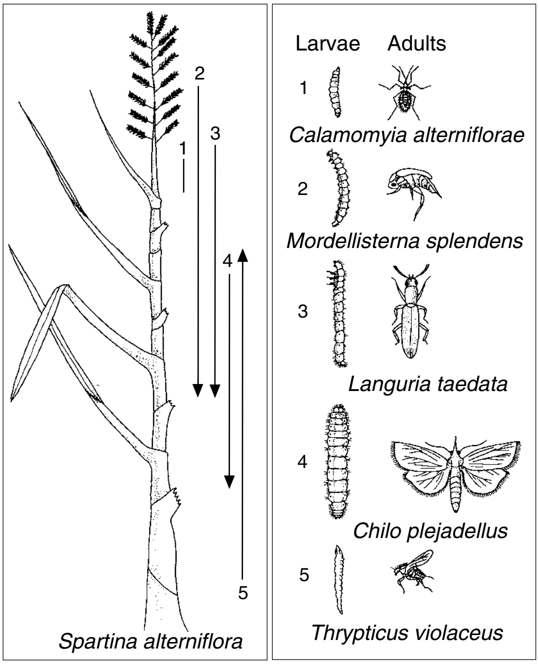


Figure 2.11 Stem-borers associated with the saltmarsh grass *Spartina alterniflora*. At different phenological stages of the plant, different borer species occupy different parts of the stem as indicated by the numbered lines to the right of the grass stem. (From Strong *et al*., 1984, with permission.)[113]

parts.[2] Nutritional factors are not, of course, the only determinants of feeding site specialization, which is evinced by almost all herbivorous insect species. Several other physiological and ecological factors must also be involved, as will be shown in chapters to follow.

2.3.2 Below-ground herbivory

Recent scientific data suggest that the total biomass of the life beneath our feet is much more vast than all that we observe above ground. Plant roots form a substantial element of this unseen world, and so do insects. The subterranean life of forests and grasslands consists typically of, among others, 100 000 to 500 000 insects and other arthropods per square metre. A considerable number of them feed on plant roots, and intimate interactions between insects and plants are likely to mirror the above-ground relationships. Some root feeders live in the soil, for example grubs that eat the smaller rootlets. Others bore directly in the roots (e.g. larvae of onion flies, carrot flies, and cabbage root flies), whereas certain cicadas and some aphid species pierce the roots and imbibe their liquid food.[130]

Root damage may result in inadequate uptake of water, nutrients, and minerals, and thereby reduce the growth of above-ground plant parts[72] and, when severe, yield losses in crop plants. Root-infesting insects may also affect their above-ground counterparts (and vice versa) via changes in their host's chemistry or physiology.[121] For instance, by damaging the roots of rice plants, rice water weevils (*Lissorhoptrus oryzophilus*) markedly reduced the growth rate of fall armyworms (*Spodoptera frugiperda*) feeding on the leaves of the attacked plants. Reciprocally, severe defoliation by fall armyworms had a negative effect on the performance of rice water weevils.[118] Herbivore damage to roots may also affect indirect plant defence. Cotton plants (*Gossypium herbaceum*) exposed to root-feeding wireworms (*Agriotes lineatus*) increased their extrafloral nectar production 10-fold in comparison to control plants with their roots intact. Extrafloral nectar recruits predators such as ants, which in turn protect the plants against above-ground insect herbivores.[124]

Because root herbivory occurs inconspicuously, it has received less attention than insect feeding on above-ground plant parts, although there is increasing evidence that root herbivores can have strong and hitherto often underestimated impacts on plant fitness.[10]

Host specialization, as found in herbivores feeding above ground as well as below ground, thus appears to have two dimensions: host-plant species and host-plant part. Only through the combination of these two features have insects evolved an abundance of species unsurpassed by other animal groups. It is remarkable that the mechanisms underlying host-plant specialization have been studied in much greater detail than the factors that restrict insects to certain plant parts only.

2.4 Number of insect species per plant species

The number of herbivorous insect species, even at a conservative estimate, exceeds the number of vascular plant species (see Fig. 2.1). As insects, except for strictly monophagous species, occur on more than one plant species, each plant may be expected to harbour several different insect species, as is readily observed to be the case in nature. Different insect species living on the same plant are not necessarily direct competitors. In addition to spatial separation, as discussed above, they are often also temporally separated because of differences in phenology between insects. The stinging nettle *Urtica dioica*, for instance, is the host plant of eight insect species, which, because of different life-cycle patterns, show seasonal differences in population build-up. As a result there is only limited overlap of population peaks between the different species (Fig. 2.12).[27]

Some plants house a larger insect fauna than others. Tansy, for example, is known to be fed upon

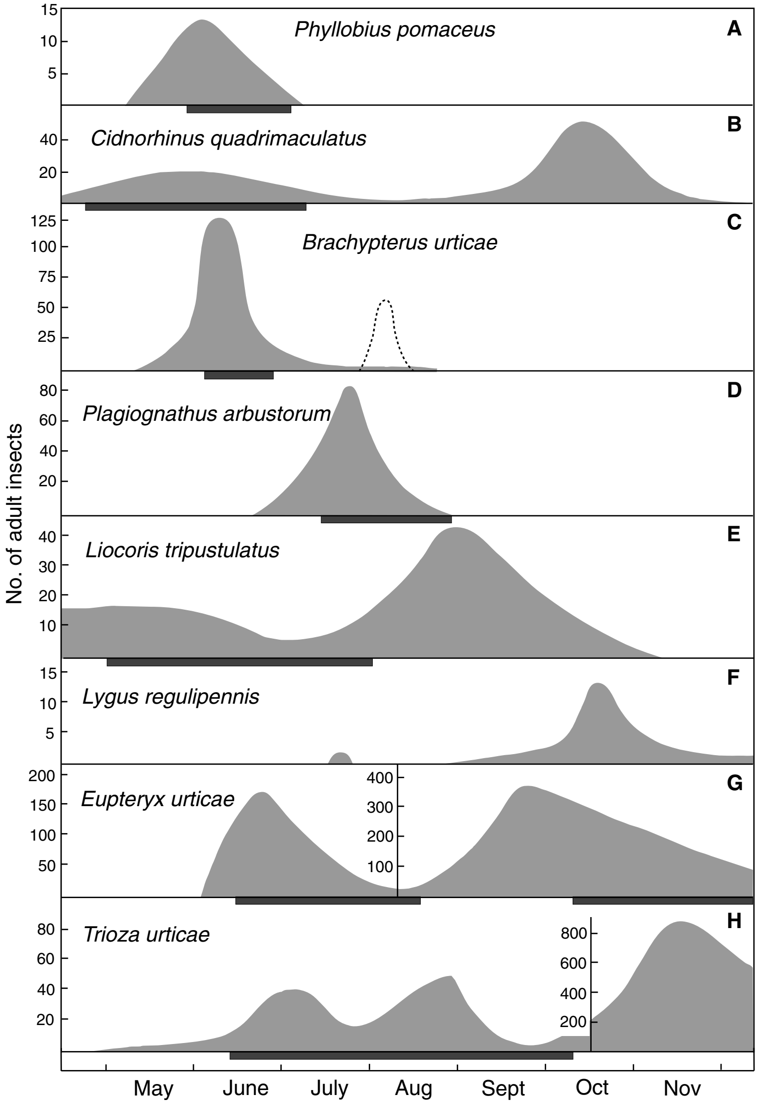


Figure 2.12 Life-cycle patterns of three Coleoptera (**A**–**C**), three Heteroptera (**D**–**F**), and two Homoptera (**G,H**) that feed on stinging nettles, as determined from weekly samples of adult insects. Thickened lines indicate the presence of adults with eggs. (From Davis, 1983.)[27]

Table 2.5 The herbivorous insect assemblage of tansy (*Tanacetum vulgare*) classified according to different feeding strategies (data from Schmitz 1998)[98]

	n	%
Total no. of species	143	
Monophagous	19	14
Oligophagous	64	46
Polyphagous	57	41
No. of tissue feeders	89	62
No. of sucking species	54	38
Local, incl. parenchyma	28	20
Phloem	23	16
Xylem	3	2
Ectophagous	92	64
Endophagous	51	36

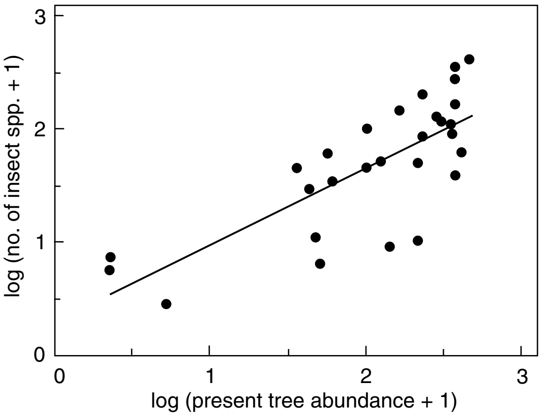


Figure 2.13 Relationship between tree abundance in Great Britain and the number of insect species inhabiting different tree species. (From Kennedy and Southwood, 1984.)[62]

by 143 different species (Table 2.5). At least 110 species, although not all herbivorous, are associated with the stinging nettle; 31 of them are specialized feeders on this plant.[27] Some 423 insect species are found to feed on two species of oak. By contrast, yew (*Taxus baccata*) supports only six insect species.[62] Of course, all plants are continuously visited by a multitude of herbivorous insect species, but only a small fraction of these visitors establish a permanent relationship with the plant. For example, Kogan has recorded the presence of more than 400 different herbivorous insects in soybean fields in Illinois, USA, but actual records of colonization are limited to no more than about 40 species.[65]

Striking differences between the number of insect species associated with different plant phyla appear when ferns are compared with angiosperms. Ferns, although evolutionarily much older than flowering plants, have on average a 30-fold lower ratio of insect to plant species than angiosperms.[20] Conceivably, the data on the insect fauna of ferns have been undercollected in comparison to those of angiosperms. More probably, however, the underpinning of the dramatic difference in the richness of insect species of both plant groups must be sought at the physiological and/or ecological level.

The differences in the numbers of insects associated with particular plants have been attributed to differences in, among others, plant life history, plant abundance (Fig. 2.13), evolutionary duration of cohabitation, plant size and architecture, and efficacy of defence mechanisms.[12,62] The relationships between these factors and the numbers of plant denizens are discussed in Chapters 10 and 11.

The foregoing discussion should not, however, give the impression that all insect species feeding on a particular plant species can readily be found on that plant. On the contrary, as most insect species are rare, the average frequency of temperate-zone herbivorous insects is around one individual per insect species per 10 or even more specimens of a particular plant species. Samplings in the tropics indicate even lower frequencies.[91]

2.5 Herbivorous insects: are they plant taxonomists?

The phenomenon of host-plant specialization requires that an insect must be able to search for and recognize its specific host even when this plant is growing in the middle of a species-rich vegetation. J.H. Fabre, in one of his famous books on insect behaviour, concluded that ovipositing females possess a 'botanical instinct' that helps them to recognize their host plants.[33] This term has also been used in a slightly different connotation to indicate that an oligophagous insect is in some way or other able to recognize the taxonomic relationship of plants, enabling it to accept only related plant species.[100] When the leaf-hopper *Aphrophora alni* was tested for its feeding preferences when exposed to eight of its normal host plants, it appeared that the insect classified the plant species in the same order with regard to genus and family as plant taxonomists.[84] The 'botanical instinct' of some specialized feeders has in some cases helped botanists to track down mistakes in earlier plant classifications. Thus, the larvae of *Thyridia* sp. were found to feed on *Brunsfelsia* spp. (Scrophulariaceae). When taxonomists realized that all known *Thyridia* species live on solanaceous plants, the taxonomic position of *Brunsfelsia* was reinvestigated, leading to transfer of the genus to the Solanaceae.[55] Several other examples have been reported in which the feeding habits of specialized insects have provided clues to taxonomic relationships between various plant taxa.[114] Thus, aphids and psyllids have been utilized successfully to solve problems in plant systematics or to distinguish closely related plant species (e.g. in the *Populus* complex), that have been confused by human botanists.[30,56] In an examination of two cottonwood species (*Populus fremontii* and *P. angustifolia*), their hybrids, and complex backcrosses, the level of concordance between a genetic analysis and a classification based upon associated herbivores was 98%. This result exemplifies that the use of insect bioassays may be a more rigorous method of distinguishing closely related plant taxa than reliance solely on, for instance, morphological or chemical characteristics.[1,37]

The above observations might lead to the conclusion that monophagous and oligophagous insects are brilliant botanists that, aided by a mysterious 'botanic instinct', unerringly recognize taxonomic relationships in the plant world. Our present knowledge of phytochemistry, however, can to a large extent explain the insect's capacity to recognize related plants, because taxonomic relationships are often synonymous with biochemical relatedness. Insects do not search for plants that have been classified by us into a particular taxon, whether it be species, genus, or family, but hunt for plants with a chemical profile that fits their search image. This profile may be rather narrow and specific and restricted to plants belonging to a single species, or somewhat broader and more variable and so characteristic of a plant genus or even family. With this explanation we have touched upon a central theme in the study of insect–plant relationships: the chemical constitution of a plant is the prime factor in its interaction with the insect world. Obviously this aspect must be discussed in much more detail; this is done, in particular, in Chapters 4–7.

2.6 Host plant is more than food plant

The host plant is not merely something fed on, it is something lived on. This statement by J.S. Kennedy[63] recognizes the importance of housing facilities provided by the host plant: biotic and abiotic factors other than food. Insects living on a plant are confronted with many kinds of cohabitant, including competitors and natural enemies, a specific microclimate, effects induced by host-plant pathogens, etc.

For instance, larvae of *Platyprepia virginalis* collected from hemlock, one of the host plants of this generalist species, appeared to be parasitized by a tachinid fly in 83% of all cases, whereas only 50% of the caterpillars collected from lupin in the same habitat were parasitized.[31] If the heavy toll taken by the parasitoid on hemlock is not compensated by some physiological or ecological advantage, the insect may be expected to develop an avoidance reaction to this host, which, although nutritionally equivalent to lupin, is suboptimal in terms of risk of parasitization.

Insects may even prefer host plants that are nutritionally suboptimal but are not visited by some of their natural enemies and thus provide an 'enemy-free space'. Such plants present better overall survival rates than more nutritious hosts where the herbivore is more vulnerable to parasitization.[79,86,106] An analysis of multispecies herbivore complexes and their host-plant assemblages likewise indicated spectacular differences in parasitism levels between forest trees belonging to 17 different plant genera.[69] These studies suggest that the strong influence of host plant on the risk of attack by parasitoids is a potentially important selective force in the evolution of herbivore diet breadth.

The observed differences in parasitism levels between different plant taxa may result from, among others, plant morphological differences, such as the presence or absence of trichomes, or plant hairs. This feature, as well as other plant characteristics, may seriously affect the efficacy of members of the third trophic level, that of insect parasitoids and predators, as is discussed in Chapters 3 and 10.

An example of a generalist herbivore feeding on physiologically suboptimal plants to reduce its mortality rate after parasitization is presented by the arctiid *Grammia geneura*. Its larvae feed preferentially on a mixture of plant species, and were found to include in their diet certain plant species that are nutritionally inferior, but provide chemicals that increase their survival chance after parasitization.[103]

From the examples given above, we may conclude that host-plant preferences are governed not only by nutritional quality, but also by environmental factors.

2.7 Microclimates around plants

A plant provides a unique microclimate for its commensals. This microclimate can differ considerably from standard meteorological measurements to which the vegetation as a whole is exposed. Plant surfaces have boundary layers of relatively still air where, because of frictionary drag, turbulence does not occur. Here temperature and relative humidity, partly as a result of photosynthetic and transpiration processes, can differ markedly from ambient levels. Moreover, probably relatively high levels of monoterpenes occur in the boundary layer due to emissions through the cuticle even when stomata are closed.[81] Although the gradients in leaf boundary layers span only millimetres, or at best a few centimetres (Fig. 2.14), depending on wind velocity and leaf size, they may be very important for any insect living in these zones. Leaf boundary layer thickness is dependent on laminar and turbulent air flows, as well as on leaf size and structure.

The upper surface of a leaf may be warm or cool depending on the rate of transpiration, its size, shape, reflectance, and height above the ground. The undersurface of a leaf is usually cooler and more humid than the upper surface. The temperature at the leaf surface may be up to 10°C, and even more, above or below the air temperature (Fig. 2.15; Table 2.6) and, likewise, the relative humidity close to the leaf surface may considerably exceed that of the surrounding air. As a consequence small insects such as aphids and early instars of folivorous insects inevitably experience microclimatic conditions that significantly influence their temperature and water balance, two basic factors of their physiology.[129]

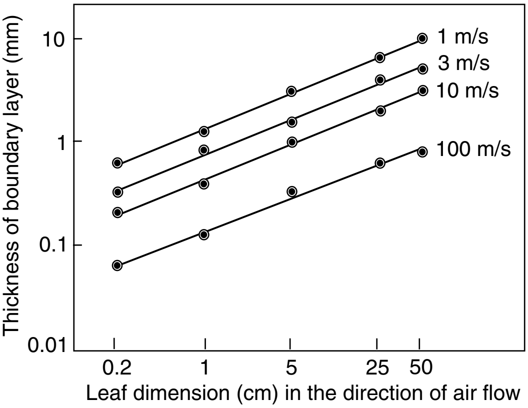


Figure 2.14 Thickness of the boundary layer over a leaf as a function of wind velocity and leaf size. Note that both axis scales are logarithmic. (From Fitter and Hay, 1987;[36] data from P.S. Nobel.)

Microclimates may also be studied at the level of whole plants or within natural vegetations (Fig. 2.16) or field crops. The microclimate at the ground surface under a vegetation differs greatly from that at a bare soil surface. When it is realized that the total surface of vegetation growing in a meadow is some 20 to 40 times the area of the ground on which it grows,[43] one can easily appreciate the effect of vegetation in reducing the amount of radiation that reaches ground level. The vegetation also produces gradients of windspeed, temperature, and humidity (Fig. 2.17).

Thus, an insect, such as the aphid *Rhopalosiphum padi*, that lives on the inflorescences of tall grasses is exposed to environmental conditions that are totally different from the microclimate experienced by the aphid *Therioaphis trifolii*, which is situated at the underside of clover leaves, although the two insects may live only a few tens of centimetres from each other.

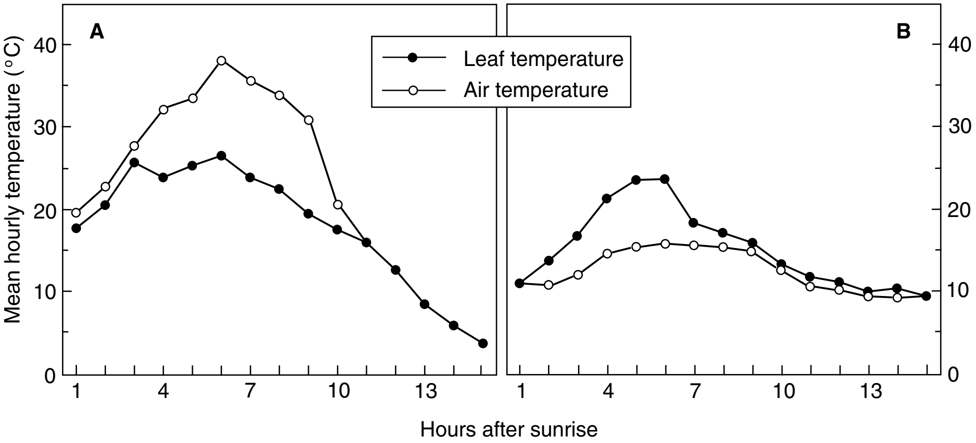


Figure 2.15 Undersurface temperatures of apple leaves and ambient temperatures measured on (**A**) a hot, cloudy summer day and (**B**) a cool, clear day. (From Ferro *et al.*, 1979.)[34]

Table 2.6 Leaf temperatures (Δ*t*) above or below air temperatures of plants from some temperate, tropical, and desert regions (data from Stoutjesdijk and Barkman, 1992)[112]

Species	Locality	Air temperature (°C)	Δ*t*
Ligustrum vulgare	Netherlands	24.3	9.5
Convolvulus arvensis	Netherlands	18.1	14.2
Rhododendron javanicum	Java, Indonesia (1500 m)	21.8	9.1
Saccharum officinarum	Java, Indonesia (lowland)	31.5	3.1
Citrullus colocynthis	Sahara desert	50.0	−13.0

2.8 Extent of insect damage in natural and agricultural ecosystems

Students of insect–plant interactions are confronted with the paradoxical observation that most plants in natural ecosystems show little or even no obvious damage despite the existence of an innumerable number of herbivorous insect species. Complete defoliation of vegetation happens only sporadically. It is estimated that insects consume in the order of 10% of all annually produced plant biomass.[4,21,26] This figure, of course, varies considerably with vegetation type, time, and locality. For instance, herbivore pressure is much more intense in tropical dry forests (average 14%) than in temperate forests (7%).[19]

Losses to sap-feeding insects are more difficult to measure, but it is estimated that they may amount to 5% or more of net primary production (NPP). Although phloem-feeders tend to be smaller in body size, they appear to consume more per gram of body mass than leaf-chewers. As a result phloem-feeders may on the whole remove as much biomass as the leaf-chewing species.[19] Occasionally their share may even be higher. This appeared, for instance, to be the case in an early successional deciduous forest, where the loss of photosynthates to sap-feeding insects greatly exceeded measured foliage loss to folivorous insects.[101]

Measuring the intensity of herbivory is often difficult.[19,101] Estimates of losses due to herbivory can differ 2–5-fold among methods used. Therefore, figures obtained for different herbivore–plant associations are in many cases difficult to compare. Some examples of damage levels for single plant species are given below to provide a general orientation, but the reader is referred to some excellent reviews for more information.[19,88,101]

When two *Eucalyptus* tree species were protected by insecticide treatment from two sap-feeding coreid bugs, the two tree species showed during a 12-month sampling period an 8.5% and 39% height advantage, respectively, compared with unprotected trees.[5] Whereas Australian *Eucalyptus* trees

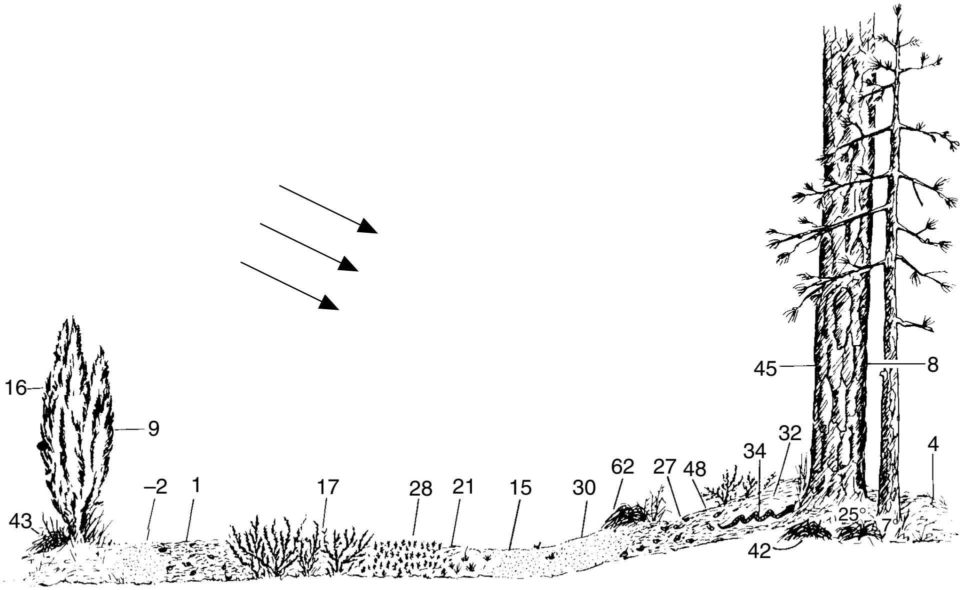


Figure 2.16 Microclimatic variations in relation to sun position and type of vegetation. Surface temperatures on a cloudless day (The Netherlands, 3 March 1976, noon) along a transect perpendicular to the fringe of a forest facing south. Air temperature at a height of 1 m was 11.8°C. The ground was still frozen in the shadow of a *Juniperus* bush, whereas a few metres to the right surface temperatures of up to 62°C were recorded. Arrows denote the direction of sun rays. (From Stoutjesdijk and Barkman, 1992.)[112]

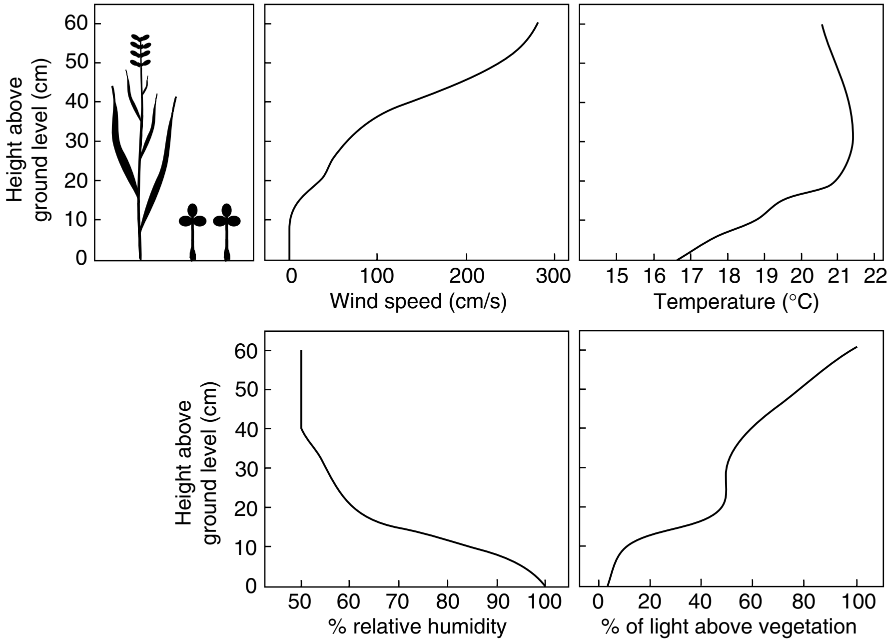


Figure 2.17 Microclimate variables in a grassland vegetation. (From Cox *et al.*, 1973.)[23]

suffer from chronic levels of insect damage, accounting for 10–50% of foliage production,[71] other plants (e.g. neem trees (*Azadirachta indica*), *Juniperus* spp., and *Rhododendron* spp.) show hardly any losses to insects. Even related plant species may show a considerable interspecific variation of losses to herbivory (Fig. 2.18).

The loss of forest trees to insects is considerable on an annual basis. For the USA and Canada it has been calculated to amount to as much as 14% and 22%, respectively.[48] More severe impacts do occur occasionally, such as widespread defoliation and death of birch forest in Fennoscandinavia caused by moth species belonging to the genera *Oporinia* and *Operophtera*.[115] Clearly, forest insect pests are of major importance worldwide and losses will probably remain high in view of the tendency to restrict the use of pesticides. Interestingly, trees in urban and ornamental plantings do not sustain more insect damage than trees in natural forests.[83]

The question arises of whether the 10% damage level represents a negligible loss of energy to a plant and, consequently, whether it significantly affects the plant's fitness. Several indications point to marked effects even at low levels of insect damage. For instance, it has been calculated that the annual net assimilate devoted to reproduction ranges from 1% to 15% in herbaceous perennials and from 15% to 30% in herbaceous annual plant species.[49] Hence, as a rough generalization, a value of 10% may cover the proportion of biomass that plants allocate to reproduction.[77] Thus, as a very general approximation, losses to insects are of the same magnitude as the energy that plants devote to reproduction. In view of the magnitude of these figures, it seems unlikely that insect damage is negligible. Of course, the 10% loss to herbivory is not the 10% spent on reproduction, as the losses are presumably shared more or less evenly by all functions.

An interesting study on oak trees showed that even moderate insect attack may markedly depress seed production. Experimental trees were regularly treated with insecticides, thereby suppressing defoliation below 5%, whereas water-sprayed control trees suffered twice that amount. Tree growth, as determined from tree rings, was not affected, but the number of acorns produced per shoot was up to four times higher in insecticide-treated trees than in untreated control trees.[25] Whether or not reduced acorn production under natural circumstances negatively affects population density of oak trees remains, however, an open question.

Sucking insects, when present in sufficient numbers, may also affect seed production negatively (Fig. 2.19). Thus, aphid-infested wood groundsel (*Senecio sylvaticus*) yields 50% fewer seeds than aphid-free plants.[32] Tree growth may also be markedly reduced by the presence of sap-sucking insects, such as scale insects. When pine trees were protected against the needle scale

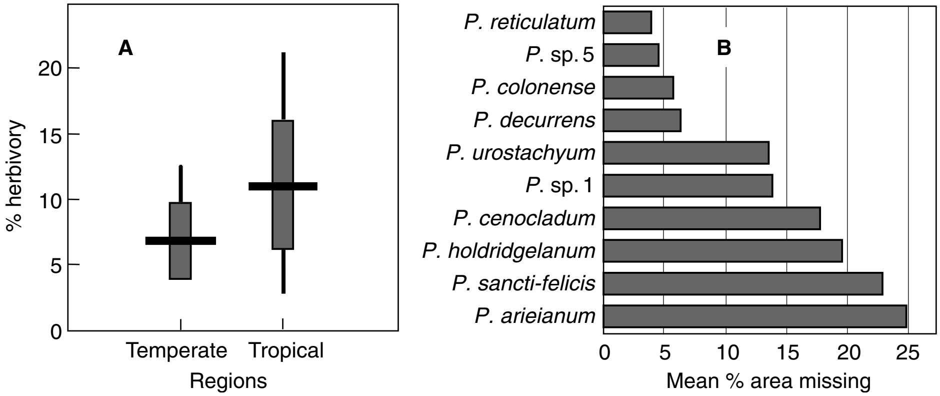


Figure 2.18 Rates of herbivory in forests in different climatic zones and for different species of *Piper*, a genus of climbing shrubs. (**A**) Leaf areas eaten annually in temperate and tropical broad-leaved forests. Plots indicate mean ± SD and range. (Redrawn from Coley and Aide, 1991).[18] (**B**) Mean percentage area missing per *Piper* species for a minimum of 50 freshly abscissed leaves per species. (From Marquis, 1991.)[75]

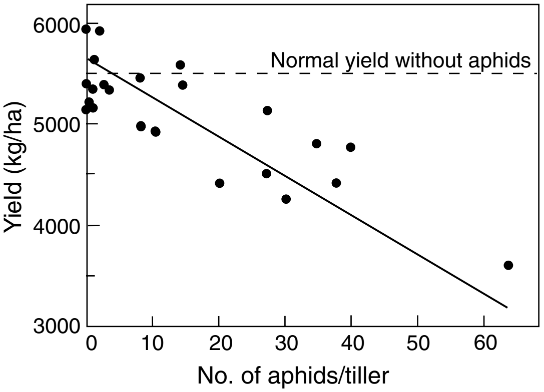


Figure 2.19 Yield of wheat in relation to peak number of grain aphids (*Sitobion avenae*) per tiller. (Data from Vereijken, 1979.)[123]

(*Matsucoccus acalyptus*), which chronically attacks at high population densities pinyon pine (*Pinus edulis*), tree ring growth increased by 25–35%.[119]

Another experimental approach to assess the effects of insect attack on seed production involves artificial defoliation. Such an experiment was done on *Piper arieianum* shrubs occurring in the neotropical rainforest that often suffer from heavy attacks by several weevil species. The plants produce fewer seeds after pruning, an effect that is carried over to the next year as a result of reduced storage allocation (Fig. 2.20). Thus, low to moderate herbivory levels often have potent effects on seed production.

Figures on losses of leaf surface possibly underestimate the real damage inflicted by insects, because many small wounds may have a much greater effect than the complete removal of some leaves, and the rate of photosynthesis may be significantly reduced in the undamaged tissue of a damaged leaf.[132] As the physiological effects of wounding are transmitted systemically to other plant parts (see Chapter 4), it is quite likely that the number of damaged sites is more important than the total size of the damaged area. When, in 12 plant species, all leaves with some signs of insect damage were scored, it appeared that on average 87% of the leaves were affected, and in some plant species all the leaves damaged to some exent (Fig. 2.21).[26] This figure is too different from the 10% damage level to be ignored.

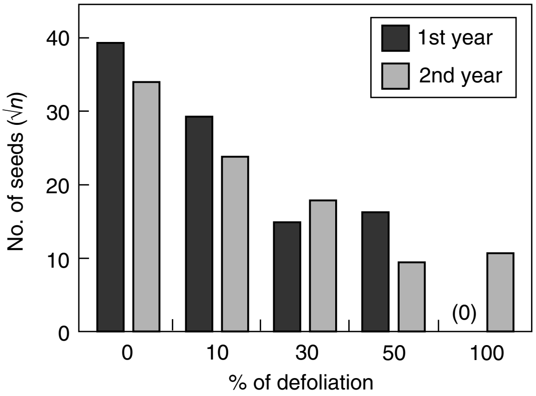


Figure 2.20 Effects of pruning on seed production by *Piper arieianum* shrubs. When 30% or more of the leaf area is removed, seed numbers are significantly reduced in the year after the treatment as well as in the following year. (From Marquis, 1984.)[74]

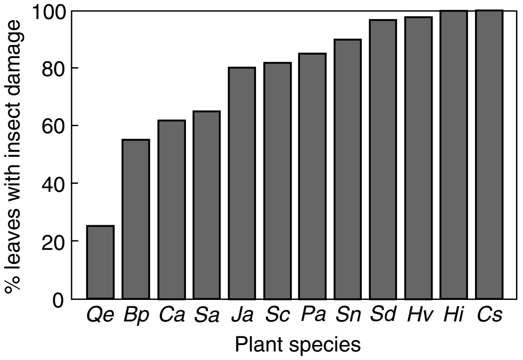


Figure 2.21 Percentages of leaves showing insect damage in 12 dicotyledonous plant species. *Bp, Betula pubescens; Cs, Calystegia sepium; Ca, Corylus avellana; Hv, Hammamelis vernalis; Hi, Heliconia imbricata; Ja, Juglans arizonica; Pa, Prunus avium; Qe, Quercus emoryi; Sc, Salix capraea; Sn, Sambucus nigra; Sd, Solanum dulcamara; Sa, Spartina altiniflora.* (It should be noted that damage assessment in many of the given species is based on only one or two plant specimens, thus shedding doubt on the representativeness of the values given.) (Data from Damman, 1993.)[26]

Other studies, however, have indicated that there are plant communities that sustain only sporadic insect damage. Price and co-workers reported that, although tropical savannah is very rich in caterpillar species, the numbers per species are very low.[91] On average, only one lepidopterous larva of all species combined was found per 10 plant individuals (1–2-m tall trees of four species).

Clearly, the intensity of insect attack may vary tremendously among plant communities and plant species, and our limited knowledge prevents us from making any sound generalization at the moment.

Insects often inflict much more damage in agroecosystems than in natural settings.[88] Despite intensive use of insecticides, crop losses to insect feeding in the USA amount to 13% (see Fig. 1.3), whereas worldwide this percentage reaches 15% or more. The phenomenon of host specialization, as discussed earlier in this chapter, has fortunate consequences for the number of pest species. About 1000 insect species attack agricultural crops in the USA. On a world scale this figure runs to about 9000 species, although less than 5% are considered to be serious pests,[89] a relatively small number in view of the many insect species present. True, our agriculture relies on a very small subset of the world's flora, with just four major and 26 minor crop species contributing 95% of human nutrition (Fig. 2.22),[96] but many of these cultivated plants have covered large areas of land for millennia and thus have offered a plethora of food to numerous insects with a notoriously high degree of adaptability. Insect pest species are also predominantly specialist feeders: 75% of temperate and 80% of tropical lepidopterous pests are monophagous or oligophagous.[3] These ratios tally strikingly with the figures presented earlier (see Section 2.1) for insect species occurring in natural vegetations.

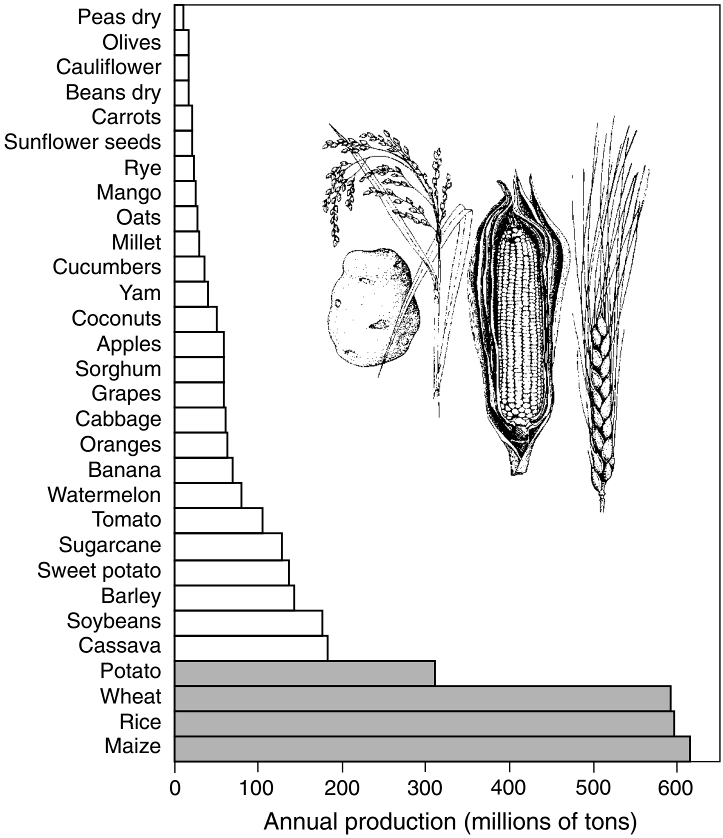


Figure 2.22 Food crop production of 30 of the Earth's 250 000 species of higher plants, which together account for 95% of human nutrition. More than 50% of our food is obtained from only four of the crop species depicted: maize, rice, wheat, and potato. (Data from Food and Agriculture Organization, 2001. < http://faostat.fao.org/faostat/form?collection=Production.Crops. >)

After discussing figures on losses of net primary production to the species-rich world of herbivorous insects, it is interesting to compare these values to the global human appropriation of NPP. Whereas humankind represents roughly 0.5% of the total biomass on Earth, humans appropriate as much as approximately 20% of terrestrial NPP.[58]

2.9 Compensation for herbivore damage

Few plants escape herbivore damage, but plants are equipped with mechanisms to reduce the deleterious effects of herbivory. As long as insects do not attack their meristemic tissues or apical tips, most plants, have remarkable powers of regeneration. From a morphological and developmental point of view, plants are basically different from animals. Structurally, plants are modular organisms, that is they consist of repetitive multicellular units each with its own meristem. None of these units is vital for the plant as a whole. This property of modularity reduces the adverse effects of herbivory considerably and allows for easy recovery from tissue removal, in contrast to unitary organisms, such as insects, that are killed or at least seriously disabled when body parts are removed. The surprising ease of recovery from herbivory results from the presence of (often dormant) meristems and the ability to redirect resources (i.e. nutrients and photosynthetic products) to regrowing tissues.[51] Under moderate or good resource conditions, plants can partially or wholly compensate, and sometimes even overcompensate, for losses to insect feeding (overcompensation being defined as the production of more biomass than has been lost to herbivory[6]). However, an unequivocal demonstration of the phenomenon of overcompensation appears to be more difficult than one might expect, because it requires that under natural conditions insect-damaged plants exhibit a significant increase in fitness as compared to undamaged controls. Many factors, including anatomical characteristics (presence of reserve meristems, vascular integration of different parts), mode of reproduction, timing of herbivory, stored reserves, and availability of water, nutrients, and light, contribute to a plant's capacity to (partially) make up for tissue losses.[29,126] As a result it is premature in the present state of our knowledge to make generalizations regarding the ecological or agricultural importance of compensatory responses (Table 2.7).

The existence of overcompensation, although demonstrated in a number of cases under growth-chamber conditions and agricultural crops,[92] remains controversial under natural field situations. A recent meta-analysis of 81 published cases of plant growth after herbivory showed that exact compensation or overcompensation occurred in 35% of the records. Surprisingly there is a difference between monocot and dicot plants with respect to optimal conditions (light, water, and nutrients) for overcompensation. Monocot herbs grew more after herbivory in high-resource conditions, whereas recovery from herbivory in dicot herbs and woody plants was significantly better in low-resource conditions.[52] This difference is probably caused by a difference in meristem location in the two groups of plants, which entails important physiological consequences.[52]

Compensation responses to insects that do not destroy the photosynthetic machinery, such as phloem sap-feeding species, are more difficult to measure. Changes in photosynthetic performance may, however, offer a clue. By measuring photosynthetic rates in holly trees (*Ilex aquifolium*) infested by scale insects (*Coccus* sp.), photosynthesis appeared to be increased compared with that in control plants, thereby compensating for losses of nutrients to herbivory. This effect extended beyond the infested leaf itself, because photosynthesis in

Table 2.7 Plant traits and environmental factors that may determine a plant's compensatory response to herbivory (from Whitham *et al.*, 1991)[126]

Undercompensation	Equal or overcompensation
Herbivory late in season	Herbivory early in season
Low water, nutrients, and/or light	Abundant water, nutrients, and light
High competition	Low competition
Meristem limitation	No meristem limitation
Slow growth	Fast growth
Non-integrated plant modules	Integrated plant modules
Woody perennials	Annuals and biannuals

scale-free leaves adjacent to infested leaves was also stimulated.[95]

Assessment of the significance of compensatory reactions in natural vegetations is obviously hampered by the fact that our knowledge of the impact of insect herbivory is derived mainly from extreme cases, whereas relatively little is known of the impact of herbivores at low densities and the relationships with effects caused by other herbivores.

2.10 Conclusions

The key point of this chapter is the high degree of food specialization generally shown by herbivorous insects. This concept will appear to be the core of all further explorations of insect–plant interactions in the remaining chapters of this book. One of the central questions raised in this area of research relates to the observation that herbivorous insects cause relatively little visible damage to plants in natural ecosystems, despite their large number of species and astounding reproductive capacity. At the same time the omnipresence of plant-feeding insects has been repeatedly hypothesized to represent a major selective force on the evolution of plant structure and function. This notion is corroborated by recent model studies on plant population dynamics which suggest that the impact of herbivory is, via reductions in seed production, more intense than heretofore expected.[73]

Apparently, plants are generally well protected against insect attack. Elucidation of the nature of the protection mechanisms may be used to develop methods for reducing insect damage in cultivated crop plants.

2.11 References

1. Abrahamson, W.G., Melika, G., Scrafford, R., and Csoka, G. (1998). Gall-inducing insects provide insights into plant systematic relationships. *American Journal of Botany*, **85**, 1159–65.
2. Åhman, I. (1985). Larval feeding period and growth of *Dasineura brassicae* (Diptera) on *Brassica* host plants. *Oikos*, **44**, 191–4.
3. Barbosa, P. (1993). Lepidopteran foraging on plants in agroecosystems: constraints and consequences. In *Caterpillars. Ecological and evolutionary constraints on foraging* (ed. E. Stamp and T.M. Casey), pp. 523–66. Chapman & Hall, New York.
4. Barbosa, P. and Schultz, J.C. (1987). *Insect outbreaks*. Academic Press, San Diego.
5. Bashford, R. (1992). Observations on the life history of two sap-feeding coreid bugs and their impact on growth of plantation eucalypts. *Tasforests*, **4**, 87–93.
6. Belsky, A.J. (1986). Does herbivory benefit plants? A review of the evidence. *American Naturalist*, **127**, 870–92.
7. Bernays, E.A. (2001). Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annual Review of Entomology*, **46**, 703–27.
8. Bernays, E.A. and Chapman, R.F. (1978). Plant chemistry and acridoid feeding behaviour. In *Biochemical aspects of plant-animal coevolution* (ed. J. Harborne), pp. 99–141. Academic Press, New York.
9. Bernays, E.A. and Graham, M. (1988). On the evolution of host specificity in phytophagous arthropods. *Ecology*, **69**, 886–92.
10. Blossey, B. and Hunt-Joshi, T.R. (2003). Belowground herbivory by insects: Influence on plants and aboveground herbivores. *Annual Review of Entomology*, **48**, 521–47.
11. Bracken, G.K. (1984). Within plant preferences of larvae of *Mamestra configurata* (Lepidoptera: Noctuidae) feeding on oilseed rape. *Canadian Entomologist*, **116**, 45–9.
12. Brändle, M. and Brandl, R. (2001). Species richness of insect and mites on trees: expanding Southwood. *Journal of Animal Ecology*, **70**, 491–504.
13. Byrne, D.N. and Bellows, T.S. (1991). Whitefly biology. *Annual Review of Entomology*, **36**, 431–57.
14. Cates, R.G. (1980). Feeding patterns of monophagous, oligophagous, and polyphagous insect herbivores: the effect of resource abundance and plant chemistry. *Oecologia*, **46**, 22–31.
15. Chapman, R.F. (1990). Food selection. In *Biology of grasshoppers* (ed. R.F. Chapman and A. Joern), pp. 39–72. Wiley, New York.
16. Chapman, R.F. and Sword, G.A. (1997). Polyphagy in the Acridomorpha. In *The bionomics of grasshoppers, katydids and their kin* (ed. S.K. Gangwere, M.C. Muralirangan, and M. Muralirangan), pp. 183–95. CAB International, Wallingford.
17. Cock, M.J.W. (1986). *Bemisia tabaci*, a literature survey on cotton whitefly with an annotated bibliography. C.A.B. Institute of Biological Control, Ascot.
18. Coley, P.D. and Aide, T.M. (1991). Comparison of herbivory and plant defenses in temperate and tropical broad-leaved forests. In *Plant–animal interactions.*

Evolutionary ecology in tropical and temperate regions (ed. P.W. Price, T.M. Lewinsohn, G.W. Fernandes, and W.W. Benson), pp. 25–49. Wiley, New York.

19. Coley, P.D. and Barone, J.A. (1996). Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics*, **27**, 305–35.
20. Cooper-Driver, G.A.N. (1978). Insect–fern associations. *Entomologia Experimentalis et Applicata*, **24**, 110–16.
21. Coupe, M.D. and Cahill, J.F. (2003). Effects of insects on primary production in temperate herbaceous communities: a meta-analysis. *Ecological Entomology*, **28**, 511–21.
22. Courtney, S.P., Kibota, T.T., and Singleton, T.A. (1990). Ecology of mushroom-feeding Drosophilidae. *Advances in Ecological Research*, **20**, 225–74.
23. Cox, C.B., Healy, I.N., and Moore, P.D. (1973). *Biogeography, an ecological and evolutionary approach*. Blackwell, Oxford.
24. Craig, T.P. and Ohgushi, T. (2002). Preference and performance are correlated in the spittlebug *Aphrophora pectoralis* on four species of willow. *Ecological Entomology*, **27**, 529–40.
25. Crawley, M.J. (1985). Reduction of oak fecundity by low-density herbivore population. *Nature*, **314**, 163–4.
26. Damman, H. (1993). Patterns of interaction among herbivore species. In *Caterpillars. Ecological and evolutionary constraints on foraging*, (ed. N.E. Stamp and T.M. Casey), pp. 131–69. Chapman & Hall, New York.
27. Davis, B.N.K. (1983). *Insects on nettles*. Cambridge University Press, Cambridge.
28. DeClerck, R.A. and Shorthouse, J.D. (1985). Tissue preference and damage by *Fenusa pusilla* and *Mesa nana* (Hymenoptera: Tenthredinidae), leaf mining sawflies on white birch (*Betula papyrifera*). *Canadian Entomologist*, **117**, 351–62.
29. Delaney, K.J. and Macedo, T.B. (2001). The impact of herbivory on plants: yield, fitness, and population dynamics. In *Biotic stress and yield loss*, (ed. R.K.D. Peterson and L.G. Higley), pp. 135–60. CRC Press, Boca Raton.
30. Eastop, V. (1979). Sternorrhyncha as angiosperm taxonomists. *Symbolae Botanicae Upsaliensis*, **22**, 120–34.
31. English-Loeb, G.M., Brody, A.K., and Karban, R. (1993). Host-plant-mediated interactions between a generalist folivore and its tachinid parasitoid. *Journal of Animal Ecology*, **62**, 465–71.
32. Ernst, W.H.O. (1987). Impact of the aphid *Aulocorthum solani* Kltb, on growth and reproduction of winter and summer annual life forms of *Senecio sylvaticus* L. *Acta Oecologica*, **8**, 537–47.
33. Fabre, J.H. (1886). *Souvenirs entomologiques*, Vol. 3. Delagrave, Paris.
34. Ferro, D.N., Chapman, R.B., and Penman, D.R. (1979). Observations on insect microclimate and insect pest management. *Environmental Entomology*, **8**, 1000–3.
35. Fiedler, K. (1996). Host-plant relationships of lycaenid butterflies: large-scale patterns, interactions with plant chemistry, and mutualism with ants. *Entomologia Experimentalis et Applicata*, **80**, 259–67.
36. Fitter, A.H. and Hay, R.K.M. (1987). *Environmental physiology of plants* (2nd edn). Academic Press, London.
37. Floate, K.D. and Whitham, T.G. (1995). Insects as traits in plant systematics: their use in discriminating between hybrid cottonwoods. *Canadian Journal of Botany*, **73**, 1–13.
38. Foss, L.K. and Rieske, L.K. (2003). Species-specific differences in oak foliage affect preference and performance of gypsy moth caterpillars. *Entomologia Experimentalis et Applicata*, **108**, 87–93.
39. Foster, S.P. and Howard, A.J. (1999). Adult female and neonate larval plant preferences of the generalist herbivore, *Epiphyas postvittana*. *Entomologia Experimentalis et Applicata*, **92**, 53–62.
40. Frost, S.W. (1942). *General entomology*. Mc-Graw Hill, New York.
41. Futuyma, D.J. (1976). Food plant specialization and environmental predictability in Lepidoptera. *American Naturalist*, **110**, 285–92.
42. Gall, L.F. (1987). Leaflet position influences caterpillar feeding and development. *Oikos*, **49**, 172–6.
43. Geiger, R. (1965). *The climate near the ground*. Harvard University Press, Cambridge, MA.
44. Görnitz, K. (1954). Frassauslösende Stoffe für polyphag an Holzgewächsen fressende Raupen. *Verhandlungen der Deutschen Gesellschaft für Angewandte Entomologie*, **13**, 38–47.
45. Gratton, C. and Welter, S.C. (1998). Oviposition preference and larval performance of *Liriomyza helianthi* (Diptera: Agromyzidae) on normal and novel host plants. *Environmental Entomolology*, **27**, 926–35.
46. Grevillius, A.Y. (1905). Zur Kenntnis der Biologie des Goldafters *Euproctis chrysorrhoea* L. *Botanisches Zentralblatt, Beiheft*, **18**, 222–322.
47. Hamilton, J.G. and Zalucki, M.P. (1993). Interactions between a specialist herbivore, *Crocidosema plebejana*, and its host plants *Malva parviflora* and cotton, *Gossypium hirsutum*: oviposition preference. *Entomologia Experimentalis et Applicata*, **66**, 207–12.
48. Harausz, E. and Pimentel, D. (2002). North American forest losses due to insects and plant pathogens. In *Encyclopedia of pest management* (ed. D. Pimentel), pp. 539–41. Dekker, New York.

49. Harper, J.L. (1977). *Population biology of plants*. Academic Press, London.
50. Harris, M.O., Sandanayaka, M., and Griffin, W. (2001). Oviposition preferences of the Hessian fly and their consequences for the survival and reproductive potential of offspring. *Ecological Entomology*, **26**, 473–86.
51. Haukioja, E. (1991). The influence of grazing on the evolution, morphology and physiology of plants as modular organisms. *Philosophical Transections of the Royal Society*, **B333**, 241–7.
52. Hawkes, C.V. and Sullivan, J.J. (2001). The impact of herbivory on plants in different resource conditions: a meta-analysis. *Ecology*, **82**, 2045–58.
53. Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H., and Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the USA*, **101**, 14812–17.
54. Hendrix, S.D. (1980). An evolutionary and ecological perspective of the insect fauna of ferns. *American Naturalist*, **115**, 171–96.
55. Hering, M. (1926). *Biologie der Schmetterlinge*. Springer, Berlin.
56. Hille Ris Lambers, D. (1979). Aphids as botanists? *Symbolae Botanicae Upsaliensis*, **22**, 114–19.
57. Howard, J.J., Raubenheimer, D., and Bernays, E.A. (1994). Population and individual polyphagy in the grasshopper *Taeniopoda eques* during natural foraging. *Entomologia Experimentalis et Applicata*, **71**, 167–76.
58. Imhoff, M.L., Bounoua, L., Ricketts, T., Loucks, C., Harriss, R., and Lawrence, W.T. (2004). Global patterns in human consumption of net primary production. *Nature*, **429**, 870–3.
59. Janz, N. (2002). Evolutionary ecology of oviposition strategies. In *Chemoecology of insect eggs and egg deposition* (ed. M. Hilker and T. Meiners), pp. 349–76. Blackwell, Berlin.
60. Karban, R. and Agrawal, A.A. (2002). Herbivore offense. *Annual Review of Ecology and Systematics*, **33**, 641–64.
61. Kato, M. (2002). First record of herbivory on Lycopodiaceae (Lycopodiales) by a dipteran (Pallopteridae) leaf/stem-miner. *Canadian Entomologist*, **134**, 699–701.
62. Kennedy, C.E.J. and Southwood, T.R.E. (1984). The number of species of insects associated with British trees, a reanalysis. *Journal of Animal Ecology*, **53**, 455–78.
63. Kennedy, J.S. (1953). Host plant selection in Aphididae. *Transactions of the 9th International Congress of Entomology (Amsterdam)*, **2**, 106–13.
64. Klausnitzer, B. (1983). Bemerkungen über die Ursachen und die Entstehung der Monophagie bei Insekten. *Verhandlungen SIEEC X*, Budapest, pp. 5–12.
65. Kogan, M. (1986). Plant defense strategies and host-plant resistance. In *Ecological theory and integrated pest management practice* (ed. M. Kogan), pp. 83–134. Wiley, New York.
66. Lawrey, J.D. (1987). Nutritional ecology of lichen/moss arthropods. In *Nutritional ecology of insects, mites, spiders and related invertebrates* (ed. F. Slansky and J.G. Rodriguez), pp. 209–33. John Wiley, New York.
67. Leather, S.R. and Awmack, C.S. (2002). Does variation in offspring size reflect strength of preference performance index in herbivorous insects? *Oikos*, **96**, 192–5.
68. Liebhold, A.M., Gottschalk, K.W., Muzika, R.M., Montgomery, M.E., Young, R., O'Day, K. *et al*. (1995). *Suitability of North American tree species to gypsy moth: a summary of field and laboratory tests*. General Technical Report NE-211. USDA Forest Service, Randor, PA.
69. Lill, J.T., Marquis, R.J., and Ricklefs, R.E. (2002). Host plants influence parasitism of forest caterpillars. *Nature*, **417**, 170–3.
70. Lindström, J., Kaila, L., and Niemelä, P. (1994). Polyphagy and adult body size in geometrid moths. *Oecologia*, **78**, 130–2.
71. Lowman, M.D. and Heatwole, H. (1992). Spatial and temporal variability in defoliation of Australian eucalypts. *Ecology*, **73**, 129–42.
72. Maron, J.L. (2001). Intraspecific competition and subterranean herbivory: individual and interactive effects on bush lupine. *Oikos*, **92**, 178–86.
73. Maron, J.L. and Gardner, S.N. (2000). Consumer pressure, seed versus safe-site limitations, and plant population dynamics. *Oecologia*, **124**, 260–9.
74. Marquis, R.J. (1984). Leaf herbivores decrease fitness of a tropical plant. *Science*, **226**, 537–9.
75. Marquis, R.J. (1991). Herbivore fauna of *Piper* (Piperaceae) in a Costa Rican wet forest, diversity, specificity, and impact. In *Plant–animal interactions. Evolutionary ecology in tropical and temperate regions* (ed. P.W. Price, T.M. Lewinsohn, G.W. Fernandes, and W.W. Benson), pp. 179–99. Wiley, New York.
76. Mattson, W.J., Lawrence, R.K., Haack, R.A., Herms, D.A., and Charles, P.J. (1988). Defensive strategies of woody plants against different insect-feeding guilds in relation to plant ecological strategies and intimacy of association with insects. In *Mechanisms of woody plant defenses against insects* (ed. W.J. Mattson, J. Levieux, and C. Bernard-Dagan), pp. 3–38. Springer, Berlin.

77. Mooney, H.A. (1972). The carbon balance of plants. *Annual Review of Ecology and Systematics*, **3**, 315–46.
78. Moskowitz, D.P. and Westphal, C. (2002). Notes on the larval diet of the painted lichen moth *Hypoprepia fucosa* Hübner (Arctiidae, Lithosiinae). *Journal of the Lepidopterists' Society*, **56**, 289–90.
79. Mulatu, B., Applebaum, S.W., and Coll, M. (2004). A recently acquired host plant provides an oligophagous insect herbivore with enemy-free space. *Oikos*, **107**, 231–8.
80. Niemelä, P., Hanhimäki, S., and Mannila, R. (1981). The relationship of adult size in noctuid moths (Lepidoptera, Noctuidae) to breadth of diet and growth form of host plant. *Annales Entomologici Fennici*, **47**, 17–20.
81. Niinemets, Ü, Loreto, F., and Reichstein, M. (2004). Physiological and physicochemical controls on foliar volatile organic compound emissions. *Trends in Plant Science*, **9**, 180–6.
82. Novotny, V., Basset, Y., Miller, S.E., Weiblen, G.D., Bremer, B., Cizek, L., *et al*. (2002). Low host specificity of herbivorous insects in a tropical forest. *Nature*, **416**, 841–4.
83. Nuckols, M.S. and Connor, E.F. (1995). Do trees in urban or ornamental plantings receive more damage by insects than trees in natural forests? *Ecological Entomology*, **20**, 253–60.
84. Nuorteva, P. (1952). Die Nahrungspflanzenwahl der Insekten im Lichte von Untersuchungen an Zikaden. *Annales Academiae Scientiarum Fennicae. Ser. A, IV Biologica*, **19**, 1–90.
85. Ødegaard, F. (2000). How many species of arthropods? Erwin's estimate revised. *Biological Journal of the Linnean Society*, **71**, 583–97.
86. Ohsaki, N. and Sato, Y. (1994). Food plant choice of *Pieris* butterflies as a trade-off between parasitoid avoidance and quality of plants. *Ecology*, **75**, 59–68.
87. Pashley, D.P. (1986). Host-associated genetic differentiation in fall armyworm (Lepidoptera, Noctuidae), a sibling species complex? *Annals of the Entomological Society of America*, **79**, 898–904.
88. Peterson, R.K.D. and Higley, L.G. (eds) (2001). *Biotic stress and yield loss*. CRC Press, Boca Raton, FL.
89. Pimentel, D. (1991). Diversification of biological control strategies in agriculture. *Crop Protection*, **10**, 243–53.
90. Potter, D.A. and Held, D.W. (2002). Biology and management of the Japanese beetle. *Annual Review of Entomology*, **47**, 175–205.
91. Price, P.W., Diniz, I.R., Morais, H.C., and Marques, E.S.A. (1995). The abundance of insect herbivore species in the tropics, the high local richness of rare species. *Biotropica*, **27**, 468–78.
92. Prins, A.H. and Verkaar, H.J. (1992). Defoliation, do physiological and morphological responses lead to (over)compensation? In *Pests and pathogens. Plant responses to foliar attack* (ed. P.G. Ayres), pp. 13–31. Bios Scientific Publishers, Oxford.
93. Robinson, A.G. (1985). *Macrosiphum* (*Sitobia*) species on ferns (revised edn). *Proceedings of the International Aphidology Symposium, Jablonna* (1981), pp. 471–4. Ossolineum, Warsaw.
94. Ruehlmann, T.E., Matthews, R.W., and Matthews, J.R. (1988). Roles for structural and temporal shelter-changing by fern-feeding lepidopteran larvae. *Oecologia*, **75**, 228–32.
95. Retuerto, R., Fernandez-Lema, B., Roiloa, R., and Obeso, J.R. (2004). Increased photosynthetic performance in holly trees infested by scale insects. *Functional Ecology*, **18**, 664–9.
96. Sattaur, O. (1989). The shrinking gene pool. *New Scientist*, 29 July 1989, 37–41.
97. Scheirs, J., Bruyn, L. de, and Verhagen, R. (2001). Nutritional benefits of the leaf-mining behaviour of two grass miners, a test of the selective feeding hypothesis. *Ecological Entomology*, **26**, 509–16.
98. Schmitz, G. (1998). The phytophagous insect fauna of *Tanacetum vulgare* L. (Asteraceae) in Central Europe. *Beiträge zur Entomologie*, **48**, 219–35.
99. Schoonhoven, L.M. (1977). Feeding behaviour in phytophagous insects, on the complexity of the stimulus situation. *Colloques Internationaux du Centre National de la Recherche Scientifique*, **265**, 391–8.
100. Schoonhoven, L.M. (1991). Insects and host plants, 100 years of 'botanical instinct'. *Symposia Biologica Hungarica*, **39**, 3–14.
101. Schowalter, T.D. (2000). *Insect ecology*, Chapter 12: Herbivory. Academic Press, San Diego.
102. Scriber, J.M. (1995). Overview of swallowtail butterflies, taxonomic and distributional latitude. In *Swallowtail butterflies. Their ecology and evolutionary biology* (ed. J.M. Scriber, Y. Tsubaki, and R.C. Lederhouse), pp. 3–8. Scientific Publications, Gainesville.
103. Singer, M.S. and Stireman, J.O. (2003). Does anti-parasitoid defense explain host-plant selection by a polyphagous caterpillar? *Oikos*, **100**, 554–62.
104. Singer, M.C., Ehrlich, P.R., and Gilbert, L.E. (1971). Butterfly feeding on lycopsid. *Science*, **172**, 1341–2.
105. Singer, M.C., Ng, D., and Thomas, C.D. (1988). Heritability of oviposition preference and its relationship to offspring performance within a single insect population. *Evolution*, **42**, 977–85.
106. Singer, M.S., Rodrigues, D., Stireman, J.O., and Carrière, Y. (2004). Roles of food quality and

enemy-free space in host use by a generalist insect herbivore. *Ecology*, **85**, 2747–53.

107. Slansky, F. and Scriber, J.M. (1985). Food consumption and utilization. In *Comprehensive insect physiology, biochemistry and pharmacology*, Vol. 4 (ed. G.A. Kerkut and L.I. Gilbert), pp. 87–163. Pergamon, Oxford.
108. Smith, R.M., Young, M.R., and Marquiss, M. (2001). Bryophyte use by an insect herbivore. Does the crane-fly *Tipula montana* select food to maximise growth? *Ecological Entomology*, **26**, 83–90.
109. Spencer, K.A. (1990). *Host specialization in the world Agromyzidae (Diptera).* Kluwer, Dordrecht.
110. Spencer, K.C., Hoffman, L.R., and Seigler, D.S. (1984). Host shift of *Ecpantheria defrorata* (Arctiidae) from an angiosperm to a liverwort. *Journal of the Lepidopterists' Society*, **38**, 192–3.
111. Stork, N.E., Hammond, P.M., Russell, B.L., and Hadwen, W.L. (2001). The spatial distribution of beetles within the canopies of oak trees in Richmond Park, UK. *Ecological Entomology*, **26**, 302–11.
112. Stoutjesdijk, P. and Barkman, J.J. (1992). *Microclimate, vegetation, and fauna*. Opulus, Knivsta.
113. Strong, D.R., Lawton, J.H., and Southwood, T.R.E. (1984). *Insects on plants. Community patterns and mechanisms*. Blackwell, Oxford.
114. Tempère, G. (1969). Un critère méconnu des systématiciens phanérogamistes, l'instinct des insectes phytophages. *Botaniste*, **50**, 473–82.
115. Tenow, O. (1972). The outbreaks of *Oporinia autumnata* Bkh. and *Operophtera* spp. (Lep., Geometridae) in the Scandinavian mountain chain and northern Finland 1862–1968. *Zoologiska Bidrag från Uppsala*, Suppl. **2**, 1–107.
116. Thompson, J.N. (1994). *The coevolutionary process*. University of Chicago Press, Chicago.
117. Thompson, V. (1994). Spittlebug indicators of nitrogen-fixing plants. *Ecological Entomology*, **19**, 391–8.
118. Tindall, K.V. and Stout, M.J. (2001). Plant-mediated interactions between the rice water weevil and fall armyworm in rice. *Entomologia Experimentalis et Applicata*, **101**, 9–17.
119. Trotter, R.T., Cobb, N.S., and Whitham, T.G. (2002). Herbivory, plant resistance, and climate in the tree ring record: interactions distort climatic reconstructions. *Proceedings of the National Academy of Sciences of the USA*, **99**, 10197–202.
120. Uvarov, B. (1977). *Grasshoppers and locusts*, Vol. 2. Centre for Overseas Pest Research, London.
121. Van der Putten, W.H., Vet, L.E.M., Harvey, J.A., and Wäckers, F.L. (2001). Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology and Evolution*, **16**, 547–54.
122. Van Nouhuys, S., Singer, M.C., and Nieminen, M. (2003). Spatial and temporal patterns of caterpillar performance and the suitability of two host plant species. *Ecological Entomology*, **28**, 193–202.
123. Vereijken, B.H. (1979). Feeding and multiplication of three cereal aphid species and their effect on yield of winter wheat. *Verslagen Landbouwkundige Onderzoekingen* 888, Centre for Agricultural Publishing and Documentation, Wageningen.
124. Wäckers, F.L. and Bezemer, T.M. (2003). Root herbivory induces an above-ground indirect defence. *Ecology Letters*, **6**, 9–12.
125. Wheeler, A.G. (1975). Insect associates of *Ginkgo biloba*. *Entomological News*, **86**, 37–44.
126. Whitham, T.G., Maschinski, J., Larson, K.C., and Paige, K.N. (1991). Plant responses to herbivory, the continuum from negative to positive and underlying physiological mechanisms. In *Plant–animal interactions. Evolutionary ecology in tropical and temperate regions* (ed. P.W. Price, T.M. Lewinsohn, G.W. Fernandes, and W.W. Benson), pp. 227–56. Wiley, New York.
127. Wiklund, C. (1975). The evolutionary relationship between adult oviposition preferences and larval host plant range in *Papilio machaon* L. *Oecologia*, **18**, 186–97.
128. Williams, M.A.J. (1994). *Plant galls, organisms, interactions, populations*. Clarendon, Oxford.
129. Willmer, P. (1986). Microclimatic effects on insects at the plant surface. In *Insects and the plant surface* (ed. B. Juniper and T.R.E. Southwood), pp. 65–80. Arnold, London.
130. Wolfe, D.W. (2001). *Tales from the underground*. Perseus, Cambridge, MA.
131. Young, O.P. (1986). Host plants of the tarnished plant bug, *Lygus lineolaris* (Heteroptera, Miridae). *Annals of the Entomological Society of America*, **79**, 747–62.
132. Zangerl, A.R., Hamilton, J.G., Miller, T.J., Crofts, A.R., Oxborough, K., Berenbaum, M.R., *et al.* (2002). Impact of folivory on photosynthesis is greater than the sum of its holes. *Proceedings of the National Academy of Sciences of the USA*, **99**, 1088–91.

CHAPTER 3

Plant structure: the solidity of anti-herbivore protection

A clear understanding of insect–plant relationships requires fairly detailed knowledge of plant structures and chemicals involved in resistance to most insects and susceptibility to others. The present chapter deals with the morphological (physical) factors that interfere with feeding or oviposition, and Chapter 4 considers plant chemicals that affect insect behaviour and physiology. The physical traits that pose a barrier or deter insects, such as leaf toughness, surface waxes, trichomes, or plant architecture, are final expressions of genetically regulated biochemical processes. Thus, morphological and chemical resistance factors intertwine in a continuum of defence.

In order properly to assess the role of some physical plant traits in insect feeding it seems opportune first to discuss briefly the major feeding systems as they occur in herbivorous insects.

3.1 Insect feeding systems

The three salient features of feeding behaviour are food choice, mode of feeding, and feeding rate. In this section we discuss the mode of feeding, whereas feeding rate and food choice are considered in more detail in Chapters 5 and 7, respectively.

Insects use one of two modes of feeding: they either bite off and chew their food,[17] or imbibe liquid nourishment.[92] Biting–chewing insects, known as *mandibulates*, possess the ancestral and more general type of mouthparts. There are three pairs of appendages, which more or less oppose one another (Fig. 3.1). The mandibles (or jaws) serve to cut and grind the food. They are equipped with tooth-like ridges to cut food and grinding surfaces to crush it. Below the mandibles are the maxillae. Each maxilla bears a segmented appendage, the maxillary palp, which is equipped with chemosensory sensilla. The maxillae aid in manipulating the food and guiding it toward the mouth. The labrum, or upper lip, forms the roof of the preoral cavity and mouth. Its ventral surface, called the epipharynx, often contains taste sensilla. The labium, or lower lip, forms the floor of the preoral cavity. It has one pair of palps bearing mechanoreceptors.

In some insect lineages the primitive mandibulate mouthparts borne on separate mouthpart regions have been converted to mouthpart types

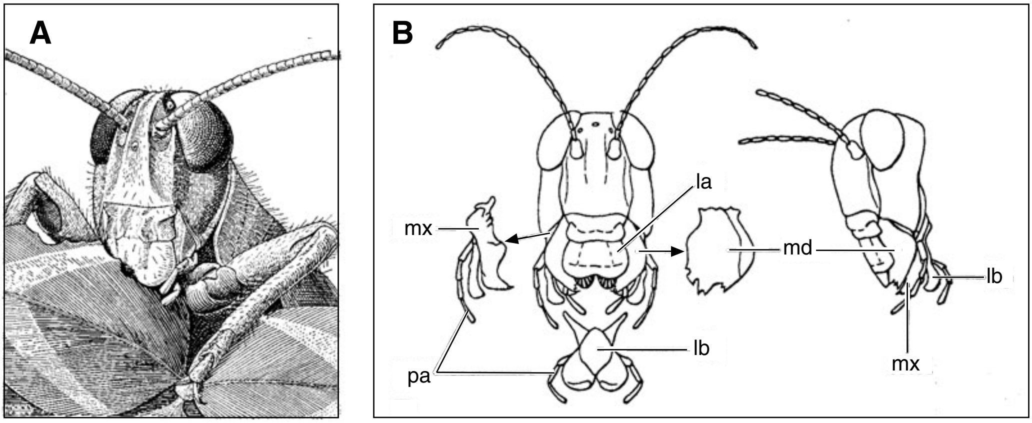


Figure 3.1 A grasshopper as an example of a mandibulate insect, feeding on clover (**A**), and frontal and lateral views of its mouthparts (**B**). Mx, maxilla; md, mandible; pa, palps; la, labrum; lb, labium. ((**A**) from Frost, 1959, with permission;[33] (**B**) from a *Textbook of Entomology* by H.H. Ross. © 1948. Reprinted with permission of John Wiley & Sons, Inc.).

consisting of functionally integrated ensembles of fused elements. They are called *haustellate* mouthparts and serve to pierce plant tissues and imbibe liquid food. Haustellate mouthparts are polyphyletic in origin, having arisen independently in Hemiptera (Heteroptera + Homoptera), Thysanoptera, and adult Lepidoptera.[58] The elongated suctorial mouthparts of butterflies and moths, and of some adult flies, consist only of the maxillae, which fit together to form a proboscis or rostrum. In the Hemiptera the labium is developed into a pronounced structure. The mandibles and maxillae are styliform and the maxillary palps atrofied. The labium is shaped into an anteriorly grooved sheath in which the two mandibular and two maxillary stylets are enclosed. In heteropteran plant-feeders its distal end is equipped with taste sensilla,[86] but aphids bear only mechanoreceptors at this location.[91] The two maxillae are interlocked in such a way that a double-barrelled tube is formed. The dorsal channel in the stylet bundle serves to take up food and the ventral one to deliver saliva (Fig. 3.2). The needle-like stylets can pierce the plant cuticle and cell walls and, once inside plant tissue, can be oriented into different directions in search of an acceptable feeding place. Further details on host-plant recognition and feeding in aphids are given in Section 7.8.8.

The food channel empties proximally into the cibarial cavity. Cibarial muscles may generate suction in the cibarium. Feeding on phloem is facilitated by the sometimes extremely high hydrostatic pressures in the sieve elements, ranging from about 0.2 to 1 MPa (2–10 atmospheres). Such high-turgor pressure in the plant's phloem can largely account for the rapid flow rates observed during aphid feeding, such as 1–2 μl/h in *Tuberolagnus salignus*.[56] Several homopterans, however, are also capable of feeding on an artificial diet lacking plant turgor pressure, or in some cases on xylem with strong negative pressures. In order to generate sufficient suction force to overcome the negative pressure, the cibarial pump in xylem feeders has an extremely well developed muscle apparatus.[56]

Sucking insects utilize several kinds of plant fluid. Many homopterans and psyllids imbibe fluid from phloem cells, whereas many Heteroptera and some Homoptera feed on the parenchyma or xylem sap. Thrips have a feeding apparatus and method of feeding that is unique among insects.[41] Several mouthparts are fused to form a mouth-cone, a short tubular base through which the actual piercing organs (two maxillary stylets and one single mandible) are protruded. Thrips live on liquids extracted from epidermal or parenchymal cells.

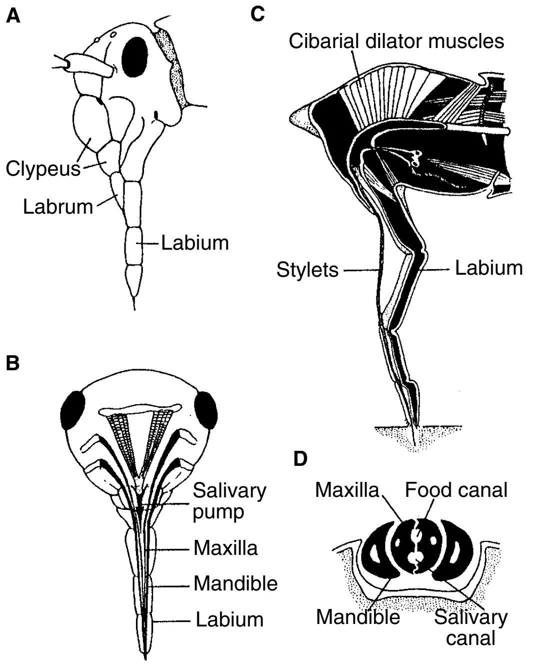


Figure 3.2 Haustellate mouthparts of Hemiptera. (**A**) Lateral and (**B**) frontal view of hemipteran head. (**C**) Longitudinal section of head and mouthparts of a pentatomid during feeding. (**D**) Schematic transverse section through stylet bundle. (From Davies, 1988, after Weber, with permission.)[24]

Many plants support both mandibulate and haustellate insect species. For instance, 335 chewers and 88 sap-feeding insect species have been recorded to feed upon two oak species.[54] The divergence in mouthpart structures allowing for the two feeding modes is an important prerequisite for food specialization and thereby for insect diversification. Obviously, both feeding methods have their advantages and disadvantages. The more delicate feeding strategy developed by sap-feeding insects places a restriction on size; sucking insects are generally smaller than chewers. They often inflict less mechanical injury on their host plants, thus exploiting their resources better than their chewing counterparts.[68] In other cases, however, sucking insects cause serious deformations and stunting of shoots, and pentatomid bugs may kill entire shoots (e.g. ears of grasses, wheat, etc.) by just a single feeding puncture (not to mention the transmission of viruses and mycoplasms by aphids and leaf-hoppers.[39,79] The direct damage inflicted by aphids is often relatively small, but the impact of spittlebugs on their host can be more severe than that of leaf-eating species.[62] Mandibulate insects, on the other hand, cannot avoid ingesting, together with nutritive compounds, large amounts of indigestible structural components of the plant, as well as toxic substances. Sap-feeders can often avoid adulteration of their food with such compounds. Phloem fluid, for instance, has a lower ratio of allelochemicals to nutrients than most other plant tissues.[80] Furthermore, phloem-feeders may derive additional protection by injecting salivary secretions into their food that detoxify some allelochemicals before ingestion.[63]

3.2 Leaf surface

The plant surface shows an enormous variety of microtextures and unicellular and multicellular outgrowths from the epidermis. These structures are, because of their small scale, usually indiscernable to the unaided human eye, but they are often of paramount importance to small herbivores and their natural enemies.

3.2.1 Epicuticular waxes

The cuticles of most vascular plants are covered with a thin layer of largely hydrophobic constituents. These wax coatings play an important role in the protection against desiccation, insect herbivory, and plant pathogen invasion. Wax layers are variable in thickness and their amount varies from a fraction of a percent to several percent of the dry weight of a plant. Moreover, wax layers are not homogeneous structures, but consist of chemically and mechanically distinct layers.[49]

Wax crystals, which show a large variety in form, emerge from a smooth and possibly amorphous wax film on all aerial surfaces (Fig. 3.3).[7,48] Several instances are known of insects that experience difficulties in feeding from leaves covered with substantial amounts of wax crystals. Thus the presence of epicuticular wax on young leaves of *Eucalyptus globulus* causes increased leaf slipperiness, thereby hindering two herbivorous psyllids from adhering to the leaf surface. As a result the survival rate of these insects was markedly reduced, possibly due

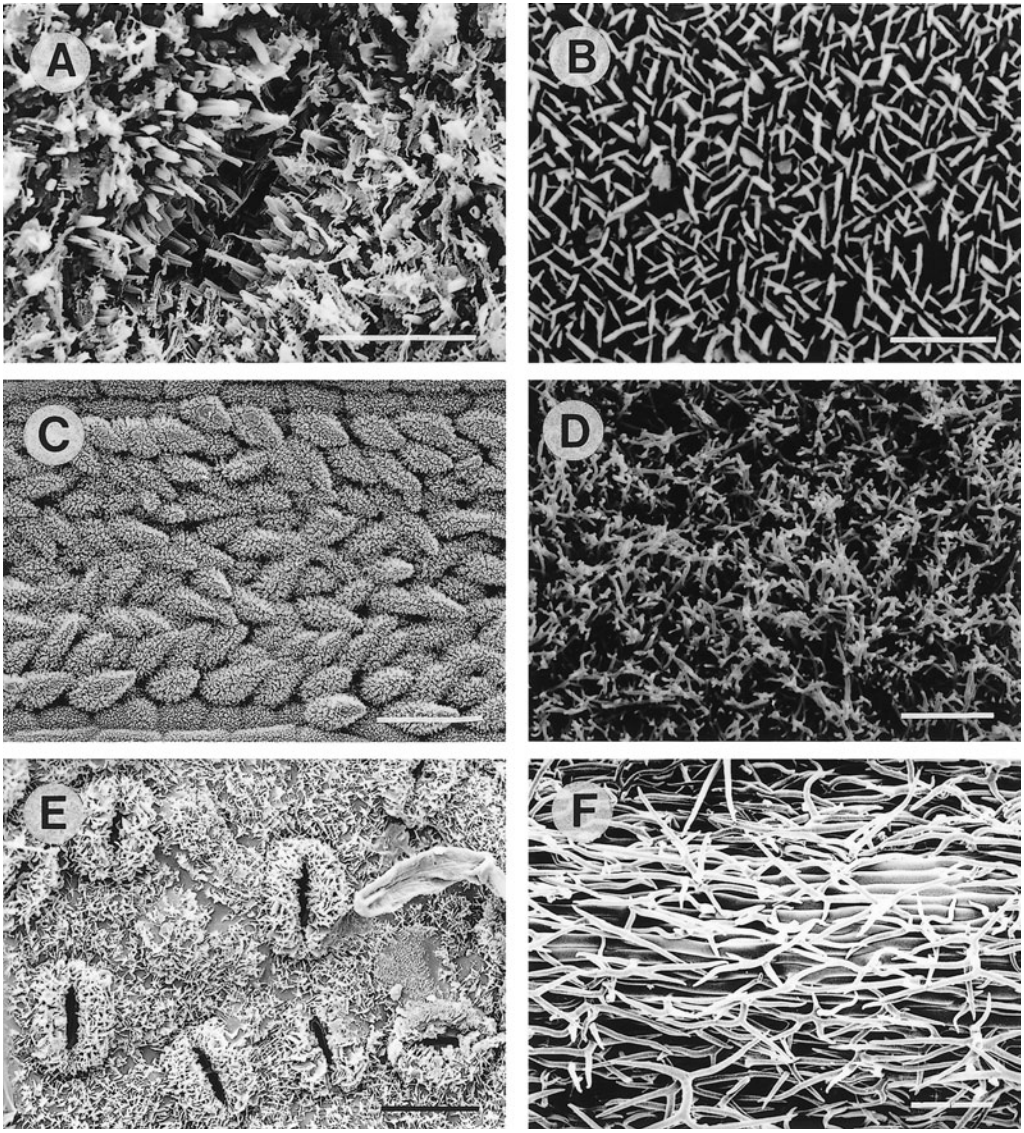


Figure 3.3 Scanning electron micrographs of plant surfaces. (**A**) *Brassica oleracea* var. capitata (Brassicaceae). Scale = 10 μm. (**B**) *Festuca arundinacea* (Gramineae). Adaxial leaf surface, showing epicuticular wax on a cell on the top of an epidermal ridge. The wax crystals are in the form of plates, typical of the crystals formed by long-chain primary alcohols. The crystals stand on edge and are mutually aligned in three preferred orientations at 120°. Scale = 2 μm. (**C**) *Cyathodes colonsoi* (Epacridaceae). Abaxial leaf surface showing a band of short, wax crystal-encrusted, epidermal trichomes overarching the stomatal complexes. Scale = 80 μm. (**D**) Sitka spruce, *Picea sitchensis* (Pinaceae). Adaxial leaf surface. Surface view of the epicuticular wax tubes, predominantly composed of *n*-nonacosan-10-ol, in the centre of the anti-transpirant wax plug that fills the stomatal antechamber. Scale = 4 μm. (**E**) *Quercus pubescens* (Fagaceae). Stomatal complexes encrusted with primary alcohol-rich wax crystals on the abaxial leaf surface. The upper (adaxial) surface lacks this thick epicuticular crust. Scale = 20 μm. (**F**) *Rosmarinus officinalis* (Labiatae). Abaxial leaf surface with dense indumentum, composed of many-branched trichomes. Scale = 100 μm. (Reproduced by courtesy of C.E. Jeffree, University of Edinburgh, UK.)

to starvation, compared with that of insects kept on 'de-waxed' juvenile leaves or adult leaves with a lower wax load.[14] Epicuticular wax bloom does not always confer resistance to insect herbivores. Several instances are known of agricultural crop varieties with reduced epicuticular wax blooms or glossy surfaces that show reduced susceptibility to insect pests (Table 3.1). Various factors have been suggested to explain this unexpected phenomenon, but as yet our insight into the mechanisms responsible for increased resistance of glossy genotypes is limited.[27]

As an indirect effect upon herbivores, dense and easily abraded microscopic wax crystals, or wax blooms, may impair the adhesion, mobility, and effectiveness of predatory insects resulting in an increase of herbivore populations. Larvae of the lacewing *Chrysoperla plorabunda*, for instance, reduced populations of *Plutella xylostella* more effectively on glossy than on normal waxbloom cabbage plants (Fig. 3.4).[29] Likewise, *C. plorabunda*,

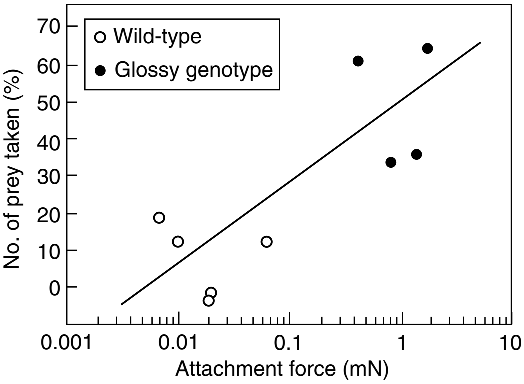


Figure 3.4 Relationship between attachment force by the predator *Chrysoperla plorabunda* to *Brassica oleracea* genotypes differing in epicuticular wax blooms, and its effectiveness at reducing populations of its prey, *Plutella xylostella* larvae. Attachment force was measured using a centrifuge to generate acceleration forces parallel to the leaf surface. (From Eigenbrode *et al.*, 1999; with kind permission of Springer Science and Business Media.)[29]

Table 3.1 Susceptibility of crops with glossy phenotypes to insect attack

Crop host	Pest insect	Effects of glossy phenotype	Beneficial trait
Allium cepa	*Thrips tabaci*	Lower infestation	Yes
Brassica napus	*Lipaphis erysimi*	Resistance	Yes
B. campestris	*L. erysimi*	Lower populations	Yes
B. oleracea	*L. erysimi*	Susceptibility	No
	Phyllotreta albionica	More susceptible	No
	P. cruciferae	Greater feeding damage	No
	Brevicoryne brassicae	Lower populations	Yes
	Erioischia brassicae	Fewer eggs	Yes
	Aleyrodes brassicae	Lower infestations	Yes
	Bemisia tabaci	Lower populations	Yes
	Thrips tabaci	Less damage	Yes
	Myzus persicae	Sometimes higher populations	No
	Plutella xylostella	Less damage, fewer eggs, lower larval survival	Yes
	Pieris rapae	Less damage and lower populations	Yes
Brassica spp.	*Phyllotreta nemorum*	Reduced leaf-mining	Yes
Glycine max	*Epilachna varivestis*	Resistance	Yes
Hordeum vulgare	Four aphid species	Higher combined populations	No
Sorghum bicolor	*Schizaphis graminum*	Less preferred	Yes
	Spodoptera frugiperda	Less damaged	Yes
	Atherigona soccata	Resistance	Yes
	Chilo partellus	Resistance	Yes
Triticum aestivum	*Sitobion avenae*	Lower populations	Yes

For references see Eigenbrode and Espelie (1995),[28] on which the table is based. (Reproduced, with permision, from the *Annual Review of Entomology*, Vol. 40, © 1995, by Annual Reviews, www.annualreviews.org)

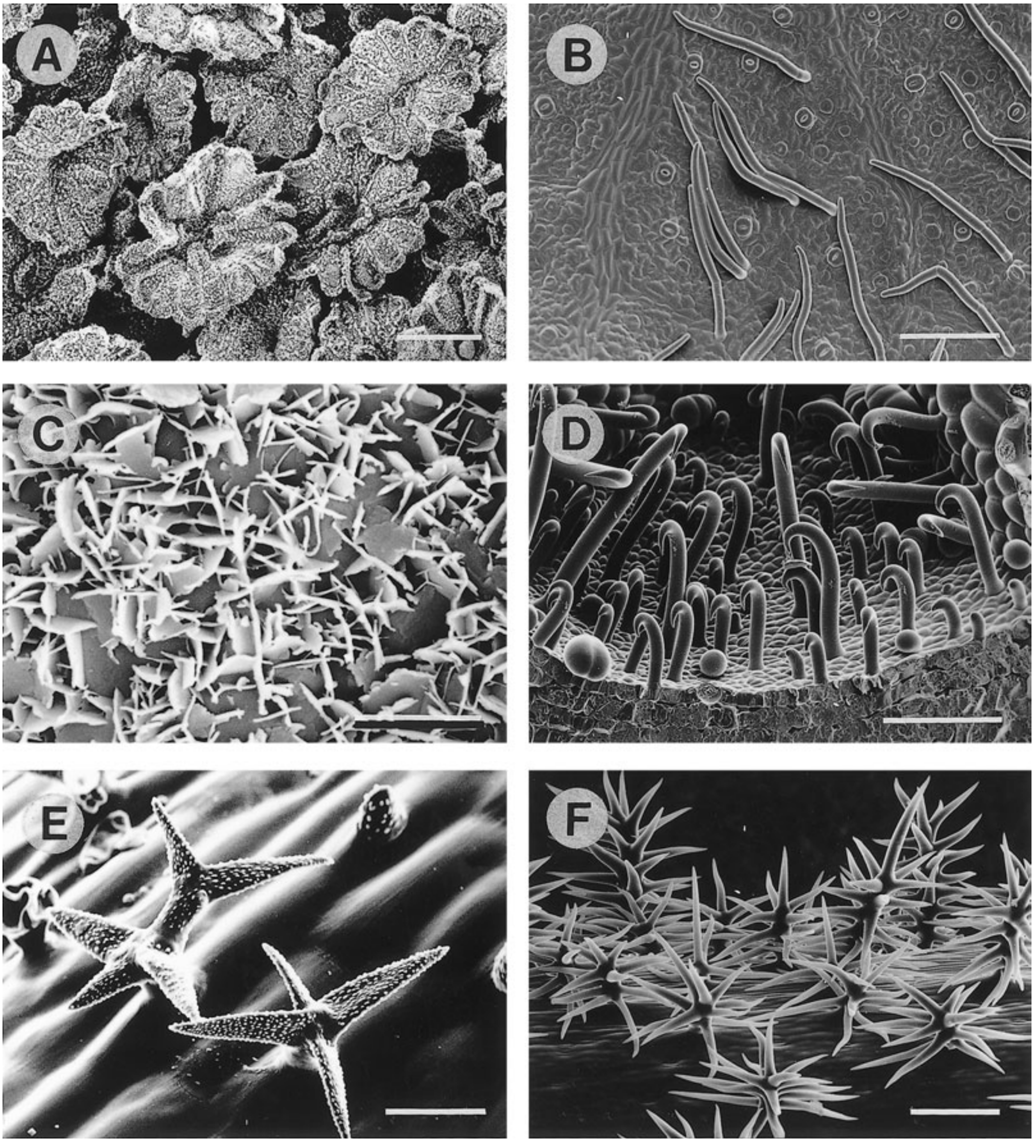


Figure 3.5 Scanning electron micrographs of trichomes. (**A**) *Rhododendron callostrotum* (Ericaceae). Waxy peltate trichomes on abaxial leaf surface. Scale = 100 μm. (**B**) Leaf of *Fagus sylvatica* (Fagaceae) that has just achieved full expansion in early summer, showing the deciduous clothing trichomes that confer a silky appearance on the expanding leaves. The epidermal cells are covered with a smooth wax film. Scale = 100 μm. (**C**) Abaxial leaf surface of *Quercus pubescens* (Fagaceae) showing detailed structure of the crystalline epicuticular wax plates. Scale = 4 μm. (**D**) Hooked trichomes on the abaxial surface of an expanding primary leaf of *Phaseolus vulgaris* (Leguminosae). The hooks catch in the tarsal joints of herbivorous arthropods, immobilizing them. Scale = 60 μm. (**E**) Branched trichomes of *Lavandula spicata* (Labiatae). The warty surface of the cells is produced by local enlargement of the cuticular layer of the cuticle. Scale = 30 μm. (**F**) Arboriform trichomes on a bud surface of kangaroo-paw, *Anigoxanthus flavidus* (Amaryllidaceae). In the young buds the dense indumentum formed by these hairs may protect them from solar radiation. Scale = 200 μm. (Reproduced by courtesy of C.E. Jeffree, University of Edinburgh, UK.)

as well as a coccinellid predator, consumed more pea aphids on pea plants with a genetically determined reduced wax bloom than on plants with a normal wax bloom.[16,96]

Getting a good grip on plants with cuticles covered with slippery wax layers presents a serious problem for many insects,[28] but several species have evolved different structures to solve this problem. Many chrysomelid beetles, for instance, have minute setae on the tarsal pulvilli excreting an adhesive material that provides good attachment to many types of smooth, hairy, and felt-like substrata.[36] Some *Empoasca* species can use their tarsal pulvilli as suction cups,[60] and many lepidopteran larvae glue a silk thread 'rope-ladder' to the plant surface to serve as a 'foothold'. Thus, various devices help insects to overcome attachment problems caused by epicuticular wax blooms on plant surfaces.[27]

3.2.2 Trichomes

Trichomes, or plant hairs, are unicellular or multicellular appendages arising from the epidermal cells of numerous plant species. They show great diversity in shape, size, location, and function (Fig. 3.5).[95] The term 'pubescence' refers to the collective trichome cover of a plant surface. A gross distinction separates non-glandular from glandular trichomes. Non-glandular trichomes frequently increase a plant's resistance to insect damage by providing an effective barrier that prevents small insects, for instance neonate larvae, from moving and contacting the plant surface. Heavy pubescence can prevent small piercing–sucking species from reaching the epidermis with their mouthparts.[88] Trichomes may also deter female insects from oviposition,[18] but in other instances ovipositing females get a better grip and therefore prefer pubescent leaf surfaces.[82] Although many cases are known in which pubescence has been found to be a resistance factor, occasionally glabrous (trichome-free) forms of plants appear to be more resistant to some insects (Table 3.2).

Laboratory studies showing a protective role for pubescence do not prove, however, insect resistance under field conditions, owing to concomitant effects on natural enemies of the pest species. Thus, trichomes may slow the searching rate of predators and parasitoids, or make the herbivore inaccessible to the point where enemies become ineffective. An example of reduced parasitoid efficacy are differences in the mortality rate of whiteflies on cucumber in relation to the presence or absence of trichomes. The minute parasitic wasp *Encarsia formosa* is considerably more efficient in finding its host, whitefly larvae, on glabrous cultivars than on hairy leaves (Fig. 3.6). On glabrous leaves, because it can move faster and manoeuvre better, this parastoid achieves parasitization levels of whiteflies 20% higher than on hairy cultivars.[94]

As is common in the insect world, some species have been able to counter the problems that trichomes may pose. The aphid *Myzocallis schreiberi*, for example, has a pair of claws and a pair of flexible empodia that help it to get a good grip on the short woolly trichomes that cover as a dense tapestry the leaves of its host, *Quercus ilex*.[53]

Another indication for a defensive role of trichomes is the intriguing observation that trichome density may vary not only with abiotic growth conditions, but also with plant damage caused by herbivory. Several annual plant species, cacti, and trees have been found to feature trichome induction in response to insect feeding.[23,93] After larvae of

Table 3.2 Role of pubescence in some selected crop plants as a resistance factor to arthropods belonging to different orders (data from Norris and Kogan, 1980,[70] and other sources)

	Resistance	Susceptibility
Wheat	C, Hy, D, Ho, D	A, D
Rice	L	
Corn	C	L
Pearl millet		L, L
Sorghum	D	
Sugarcane	Ho, Ho, L	
Soybean	C, Ho, C, D, D, D, L	L
Pigeonpea	L	
Alfalfa	Ho, C, Hy, Ho,	
Cotton	L, C, He, L, Ho, He, He, L	L, L, Co, Ho, L, Ho
Beans	Ho, Ho, Ho, Ho	L, T
Cabbage	C	L
All plants	36 insect species	15 arthropod species

A, Acarina; C, Coleoptera; D, Diptera; He, Heteroptera; Ho, Homoptera; Hy, Hymenoptera; L, Lepidoptera; T, Thysanoptera.

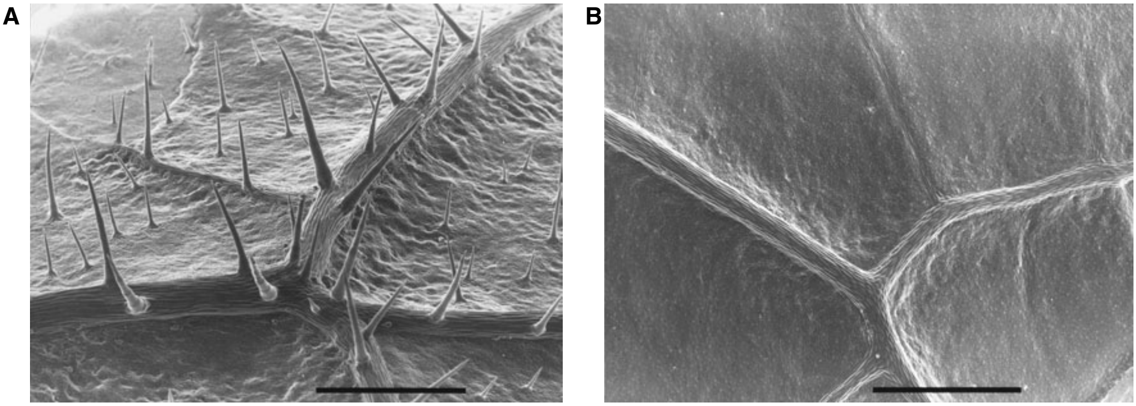


Figure 3.6 Scanning electron micrographs of the underfaces of (**A**) a hairy and (**B**) a glabrous leaf of two cucumber cultivars. The longest trichomes of the hairy leaf are about the size of *Encarsia formosa* adults. Scale = 1 mm. (Reproduced by courtesy of J.C. van Lenteren, Wageningen University, The Netherlands.)

Pieris rapae or *Trichoplusia ni* had consumed parts of young black mustard plants (*Brassica nigra*), trichome densities on some newly expanded leaves increased. Although the response depended greatly upon the herbivore and leaf position, trichome densities appeared in some instances to be more than doubled.[93]

By secreting allelochemicals, glandular trichomes may evoke toxic and disruptive effects in various types of herbivore. They thus serve in chemical protection, as discussed in Chapter 7.

3.3 Leaf toughness

Plant cell walls strengthened by deposition of macromolecules such as cellulose, lignin, suberin, and callose together with sclerenchymatous fibres make a plant resistant to mechanical injury as well as to the tearing action of mandibles or the penetration of piercing–sucking mouthparts and ovipositors of herbivorous insects. Leaf toughness, therefore, acts as an effective factor reducing herbivory, a view supported by Coley's finding that leaf toughness is the best predictor of interspecific variation in herbivory rates.[22]

3.3.1 Mandible wear

Feeding on tough plant parts often results in considerable wear of the mandibles,[17] even though the highly sclerotized mandibles of many insect species can be extremely hard, deriving extra hardness from the incorporation of zinc or manganese in their cuticle.[85] Plant leaves vary greatly in toughness and hardness. Grasses, for instance, are three times tougher than an average herb (Table 3.3).[12] In addition to differences between plant groups, there are also differences in average leaf toughness between different climatic zones. Thus, leaf toughness of tropical forest trees shows 3-fold greater values than those measured in temperate zones. This difference may (partly) be attributed to the greater selective pressure of insect herbivory in the tropics, where mean folivory amounts to 16.6% compared with 7% in temperate zones.[26]

Table 3.3 Relative leaf toughness or hardness in plants with different growth form, with leaves of herbaceous dicots standardized to 1 (from Bernays, 1991)[12]

Plant type	*N*	Relative toughness
Herbaceous dicots, all leaves	166	1.0
Woody plants, new leaves	25	1.7
C_3 grasses, all blades	42	3.1
C_4 grasses, all blades	34	6.2
Woody plants, fully expanded leaves	89	6.3
Palms, expanded fronds	8	9.8

N, number of species tested.

Differences in leaf toughness (for terminology and measuring techniques see Hochuli[43] and Sanson *et al.*[83]) probably affect insect feeding and growth more than is often thought (Fig. 3.7). The polyphagous beet armyworm (*Spodoptera exigua*), for example, takes more than three times longer to swallow food particles from celery (*Apium graveolens*) than from nettle-leafed goose foot (*Chenopodium murale*), because the leaves of celery are 1.5 times as tough as those of goose foot.[9] Likewise, there is a significant correlation between the leaf toughness of various maize cultivars and resistance to the European corn borer.[10]

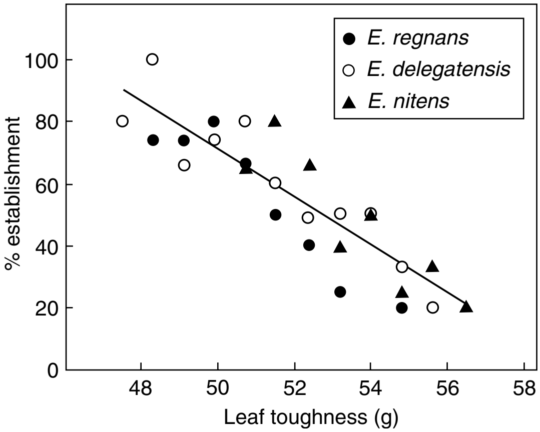


Figure 3.7 Establishment of neonate beetle larvae (*Chrysophtharta biomaculata*) depends on the leaf toughness of three of their host plants, *Eucalyptus* trees. Establishment on leaves with a toughness below 46 g was 100%, whereas on leaves above 56.5 g no larval establishment occurred. (From Howlett *et al.*, 2001.)[45]

The size of leaf fragments swallowed by chewing insect species varies with size (instar) of the insect and hardness of the food. Thus, the alimentary tract of saturniid caterpillars feeding on tough leaves contain leaf particles that are relatively large and very regular in size, whereas sphingid larvae generally feeding on the soft leaves of herbaceous hosts bite off small leaf particles independent of caterpillar size (Fig. 3.8).

As most insects digest cell walls only to a very limited degree,[44] inefficient digestion would be expected for insects with the habit of taking only large bites. This, however, is not the case. The frass of a lepidopteran (*Paratrytone melane*) contains leaf pieces with 76–86% uncrushed cells. Yet, the approximate digestibility of soluble carbohydrates and protein averaged 78% and 88%, respectively. Most likely the nutrients are extracted from the uncrushed cells through plasmodesmata and cell wall pores after the cell membranes have been digested.[4]

Within certain limits an insect may adapt its head morphometrics to the toughness of its food, as exemplified by caterpillars of *Pseudaletia unipunctata*, in which the head and chewing musculature are

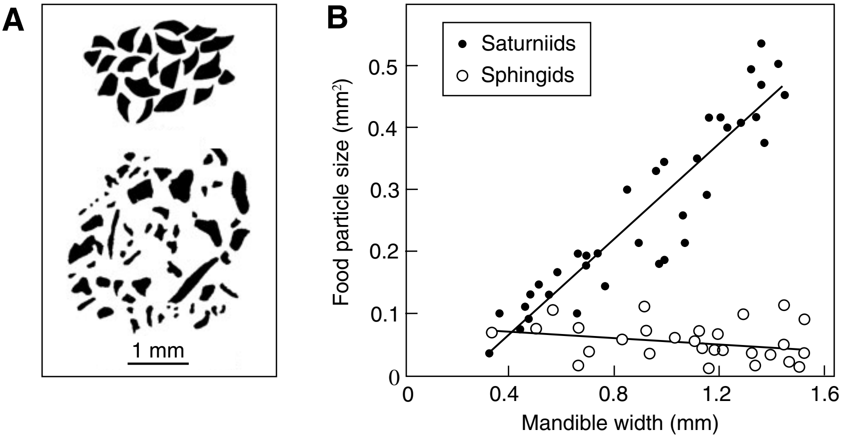


Figure 3.8 (**A**) Drawings of food particles from the midgut of (*top*) a saturniid (*Rothschildia lebeau*) and (*bottom*) a sphingid (*Pachylia ficus*) fifth instar caterpillar. (**B**) Relationship between mandible width and size of chewed food particles in the gut of 10 saturniid and 15 sphingid caterpillars (various instars). The saturniid species fed on tough and thick mature leaves, whereas the sphingids fed upon soft and flimsy leaves, both old and new. (From Bernays and Janzen, 1988.)[13]

twice as large when fed on hard grass than on soft artificial food, even though body mass is similar.[11] Water lily beetles (*Galerucella nymphaeae*) feeding on water lily have disproportionally bigger mandibles than conspecifics feeding on *Rumex hydrolapathum*, another host plant with leaf tissues about three times softer than those of water lily. It is not clear whether in this case the observed differences are genetically based or host-plant induced.[77] Furthermore, mandible morphology varies between species; this may allow the insects optimally to exploit certain types of food plant. Grasshopper species, for example, may show great differences between their mandible shape in correspondence with differences in hardness of their food plants (Fig. 3.9).

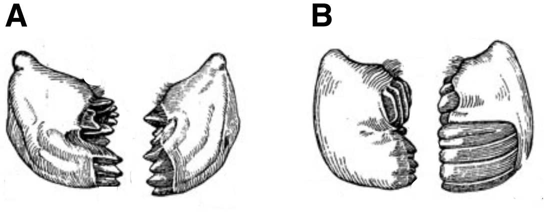


Figure 3.9 The mandibles of two grasshopper species with different diets. (**A**) *Brachystola magna* is a forb-feeding species. In chewing and grinding leafy forbs the molar denticles of the right and left jaws are interspaced so as to produce an efficient masticory mechanism. (**B**) *Mermiria maculipennis* is a prairie acridid. Its incisor dentes of the left mandible are almost fused to form a continuous cutting edge which, together with the bevelled edge of the right mandible, produces a scissor-like cutting mechanism. (From Isely, 1944.)[47]

Chewing off particles of tough plant tissues requires quite some energy and causes severe wear of mouthparts compared with feeding on softer tissues. Cellulose, an important component of cell walls, may thus act as a broad-spectrum resistance factor to insect herbivores. Wear can be especially excessive when feeding on plants with a high silicon content (up to 15% dry weight, which is more than any other inorganic constituent), such as Poaceae (grasses and cereals), Cyperaceae (sedges), Palmae, and Equisetales (horsetails). Amorphous silicon ($SiO_2 \cdot nH_2O$) particles deposited in cell walls and cell lumens[31] serve as a harsh abrasive that may cause complete loss of mandibular teeth during the feeding process, resulting in death by starvation. Increased silicon content in wheat,[66] rice,[55] sugarcane,[52] and other poaceous crop plants contributes to resistance to several insect pest species (Fig. 3.10).

Physical defence does not require that all cells or tissues of a plant have similar mechanical properties. Silicon, for instance in Poaceae, is not homogeneously distributed and collenchyma tissue renders only the peripheral leaf regions of holly (*Ilex aquifolium*) too hard to be chewed off by insects feeding at leaf edges, as most caterpillars do (Fig. 3.11).[61]

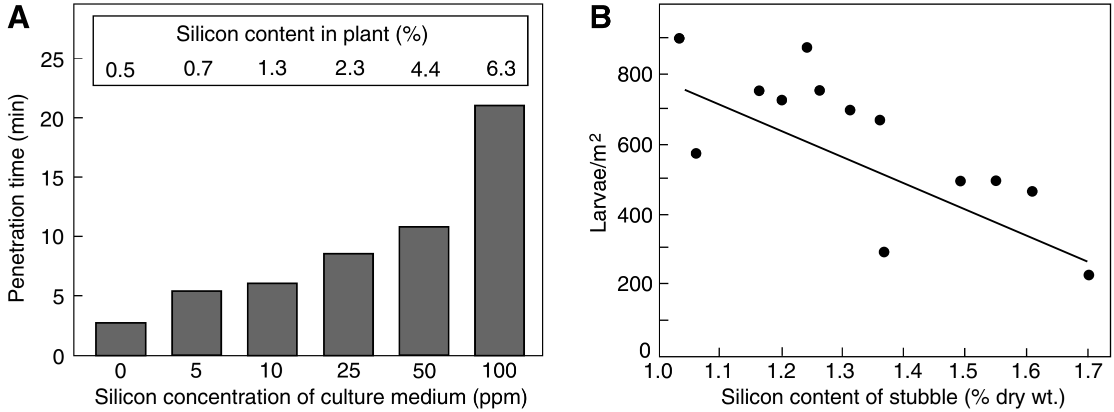


Figure 3.10 Effects of silicon concentrations on herbivore survival. (**A**) Penetration time required by newly hatched yellow stem-borer (*Scirpophaga incertulas*) larvae to enter stems of rice plants grown on nutrient solutions with different silicon levels. (From Khan and Ramachandran, 1989.)[55] (**B**) The susceptibility of 13 Italian ryegrass (*Lolium multiflorum*) varieties to attack by stem-boring frit fly (*Oscinella frit*) larvae in relation to silicon content of their stubble. (Data from Moore, 1984.)[65]

3.3.2 C_3 and C_4 plants

Based on differences in photosynthetic pathways, plants are classified as C_3 plants, which include the majority of temperate species, and C_4 plants. The photosynthetic capacity of C_4 plants is much higher than that of C_3 plants. They can reduce the concentration of carbon dioxide in their intercellular spaces far below that of C_3 plants, and as a result achieve higher photosynthetic rates at a given level of stomatal conductance, with less water loss. Their water requirements are approximately half as high as those of C_3 plants, explaining the fact that they occur predominantly in (sub)tropical and dry habitats (Fig. 3.12). Species of the C_4 type are almost exclusively grasses, favouring hot, dry growing seasons. They include economically important crops, such as maize, sorghum, millet, and sugar cane (wheat, rice, and barley are, however, C_3 plants). The C_4-type plants evolved from plants with the C_3 pathway, possibly in response to a number of environmental changes during the Miocene epoch (between 25 and 5 million years ago).[75] The differences in carbon fixation processes have important physiological as well as morphological consequences. The C_4 metabolism is accompanied by a distinctive leaf structure, termed Kranz anatomy. In these plants the veins are surrounded by a layer of large, thick-walled, vascular bundle sheath cells. These anatomical modifications have been found to affect insect herbivory. The edible sheath cells are reinforced with hemicellulose, which many insects cannot digest. Several studies have shown that herbivorous insects tend to avoid C_4 plants. Their anatomical characteristics increase their toughness (see Table 3.3), and physical constraints clearly deter many insects

Figure 3.11 Leaves of holly (*Ilex aquifolium*) have leaf edges that are too hard to be eaten by, among others, caterpillars of the oak eggar, *Lasiocampa quercus*. When the spiny margins of the leaves are cut away, the polyphagous larvae of this species will readily feed on the remaining leaf parts.

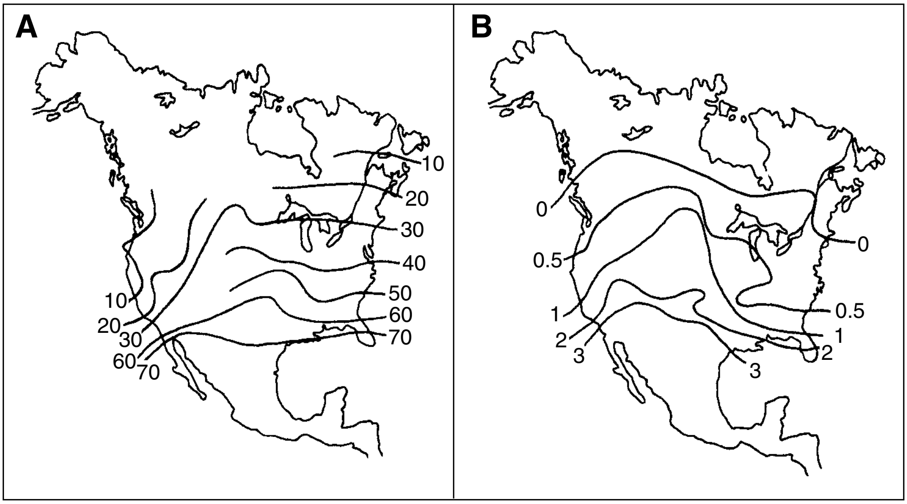


Figure 3.12 'Contour' lines indicating (**A**) the percentage of grass taxa and (**B**) the percentage of dicotyledon taxa that use the C_4 pathway in photosynthesis in North America. (From A.H. Fitter and R.K.M. Hay 1987, *Environmental physiology of plants*, 2nd edn, Academic Press, London, with permission.)

from feeding and ovipositing on C_4 plant species.[84] Alternatively, C_4 plants may harbour fewer insects than C_3 plants as a result of nutritional differences.[5,6] However, whereas there appears to be firm proof for the biomechanical explanation, the effects ascribed to differences in nutritional value are less unequivocal. Of course, the two mechanisms are not mutually exclusive.

The general tendency of C_4 plants to be less susceptible to herbivorous insects should not be interpreted as indicating that these plants are devoid of insect attackers. Thus, the economically important C_4 crops—maize, sugar cane, sorghum, and millet—do host hordes of insect pests. This may also be caused, at least partly, by intense selection for particular yield components, as well as by agricultural practices.

3.4 Structures involved in mutualistic relationships

Domatia and extrafloral nectaries are plant structures that provide shelter and food to predaceous arthropods and thus affect herbivorous insects only indirectly. Their wide occurrence among angiosperms indicates an important role in the relationships between plants and insects (Fig. 3.13). The distribution and function of extrafloral nectaries are discussed in Chapter 10.

A leaf domatium (Greek for 'little room') is a morphogenetic structure that is widespread among woody plants.[73] Domatia occur in plant species belonging to over 90 (from a total of about 420) families, often in the form of small hair tufts, pockets, or invaginations in the major vein junctions on the undersides of leaves. 'Acarodomatia', which are only 1–2 mm in diameter, offer predatory and fungivorous mites shelter to adverse climatic conditions[37] or protection from intraguild predators.[30,71] Domatium occupants often play a role in reducing the population of herbivorous mites and fungal parasites.[74] A study in which the domatia on the leaves of laureltinus (*Viburnum tinus*) were removed showed that the number of predatory mites decreased, especially under conditions of low relative humidity. Their lower density resulted in a lower predation rate on herbivorous mites.[37] Another manipulative study, in which structures mimicking domatia were applied to the leaves of cotton plants, showed that domatia-bearing plants hosted larger populations of predatory arthropods and smaller populations of herbivorous mites than control plants. This resulted in a spectacular increase in fruit production of 30% in plants with domatia.[1]

Other well known examples of domatia are hollow stems and thorns, which occur in hundreds of tropical plants, providing refuge and nesting sites to ants. Owing to their carnivorous habits, these ants effectively protect their host plant against insect damage.[46] Moreover, the presence of ant domatia also results in metabolic savings, as plant species defended by ants do not invest in such costly chemical defences as related species that do not provide housing to ants.

The examples given may suffice for the conclusion that, although high construction costs are involved in producing domatia,[34] they represent a

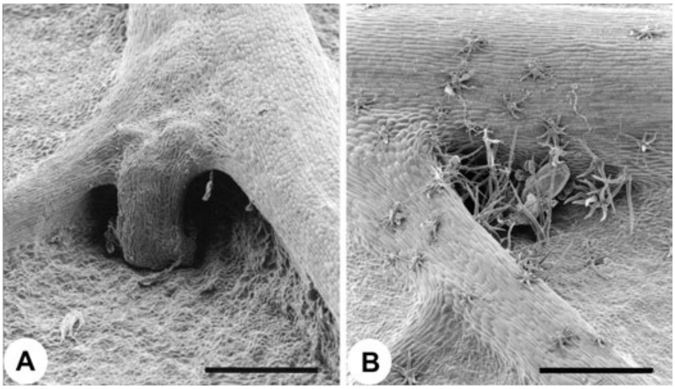


Figure 3.13 Scanning electron micrographs of the surface morphology of two leaf domatia. (**A**) *Acer tegmentosum*; (**B**) *Styrax japonica* with a phytoseiid mite. Scale = 0.5 mm. (Reproduced by courtesy of D. J. O'Dowd, Monash University, Australia.)

widespread form of morphology-based protective mutualism between plants and arthropods.

3.5 Plant galls

Some of the most exquisite modifications of plant shape are caused by galling insects.[87,97] A clear-cut definition of plant galls is difficult to formulate, because of their heterogeneity in form and causative organisms. The one given by Redfern,[81] however, seems straightforward: 'A plant gall is a growth or swelling caused by hypertrophy (enlargement) and/or hyperplasy (multiplication) of plant cells, induced by an organism, which provides nutrients/ food and shelter for that organism' (p. 55). Members of various insect orders may induce galls, but those with the greatest structural complexity and exhibiting well developed nutritive and sclerenchymatous tissue are often induced by gall midges (Diptera: Cecidomyiidae) or cynipid wasps (Hymenoptera: Cynipidae). Whereas genuine plant tumours are generally unorganized and amorphous cell complexes, insect-induced galls exhibit a distinct morphological organization as well as physiological function. The assumption that the galling insect plays an active role in determining the shape of a gall is supported by the fact that even closely related insect species may in the same host plant induce galls that differ greatly in structure and function (Fig. 3.14). For instance, whereas the tephritid *Urophora quadrifasciata* elicits a primitive achene gall in the flowerhead of *Centaurea jacea*, its congener *U. jaceana* induces a complex multilocular ovariole-receptacle gall.[15]

Not only development of gall structure, but also the movement of assimilates and nutrients to the gall, is controlled by the insect within. An analysis of the distribution of photoassimilates in dandelion (*Taraxacum officinale*), employing $^{14}CO_2$, showed that galls function as physiological sinks for photoassimilates and may drain, depending on the number of galls per plant, up to the astonishing amount of 70% of total carbon produced by the host.[3] Likewise, changes in amino acid concentrations in phloem sap occurred in leaves of *Sorbus commixta* with galls induced by a gall-inhabiting aphid species. In this tree the amount of amino acids present on exudates from cut galled leaves was five times that in ungalled leaves, probably due to the breakdown of leaf protein and to the benefit of the galling insect.[57]

Galling insects also appear to manipulate the biosynthesis of defensive compounds in their hosts, as was concluded from a study on the phenolic chemistry of willows. Galls induced by sawflies (*Eupontania* spp.) in various willow species contained substantially lower concentrations of most phenolics in gall interiors than in leaves. In some

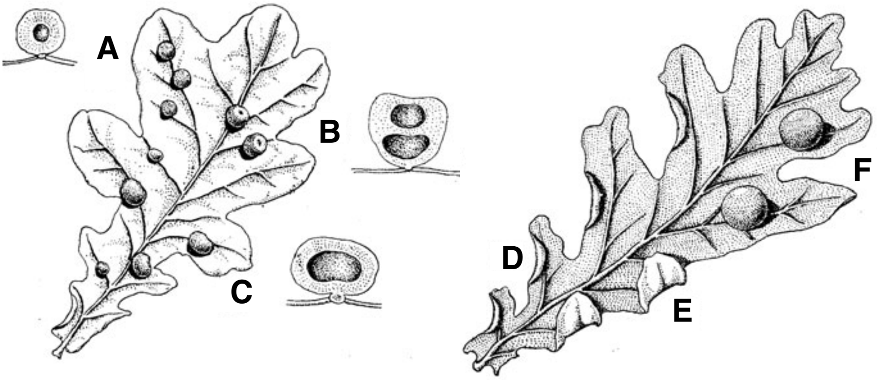


Figure 3.14 Oak (*Quercus robur*) leaves with galls from four cynipid (**A, B, C, F**) and two cecidomyid (**D, E**) wasps. (**A**) *Trichonaspis synaspis*, (**B**) *Cynips disticha*, (**C**) *C. divisa*, (**D**) *Macrodiplossis volvens*, (**E**) *M. dryobia*, (**F**) *Neuropterus baccarum*. Transverse sections of their corresponding galls for three species (**A**–**C**) to show species-specific differences. (From W.M. Docters van Leeuwen 1957, *Gallenboek*, 2nd edn, Thieme, Zutphen, with permission.)

way or other, the galler is able to change the quality and quantity of the phenolic assortment in its host's anomalous tissue.[72]

The large variety of gall forms as a function of the diversity of galling insects raises the question about the identity of the gall-inducing substances produced by the gallers and the molecular events taking place in the host plant upon infestation. Very little is known in this respect, but the isolation of some insect-derived mitogenic lipids that can stimulate gall formation in the absence of insects may present a lead to resolving these intricate insect–plant interactions.[42,89] More details about host-plant responses to elicitors produced by insects are given in Chapter 4.

Are plants wholly defenceless against galling insects? The answer is no, as many plant species are known to respond with a local hypersensitivity reaction to an infesting galler, thereby often killing the insect. The significance of such responses is demonstrated by the results of a survey of mortality rates in galling insects in eight woody plant species. This study showed that, depending on the plant species, 12–94%, (average 59%) of the invading insects succumbed as a result of hypersensitivity reactions by their host plant.[32] Apparently, plants exert a strong selection pressure on galling insects, which, together with the required evolutionary adjustments between gall inducer and host plant, would foster host-plant specialization. A narrower host breadth has indeed been found among galling lepidopterans compared with non-galling endophagous Lepidoptera.[64]

The above, necessarily limited, information allows the statement that the insect, not its host plant, determines the location, size, shape, and to some extent even the physiology of galls. Thus, the development of an insect gall, although composed of host plant tissues, is controlled largely by the insect's genes and can therefore be regarded as an 'extended phenotype' (*sensu* Dawkins[25]) of the galling insect.

3.6 Plant architecture

Plant size and architecture affect the number of insect species living on it. Clearly a moss plant will harbour fewer species than an oak tree. The term plant architecture is applied to the size and growth form of a plant, including attributes such as canopy spacing, stem, leaf, and bud shapes and dimensions, branching angles, and surface complexity (texture and pubescence) at a point in time. In a broader definition the term also encompasses a plant's seasonal development and persistence.[59] The surfeit of architectural traits impedes the development of realistic plant architecture models,[35,38] which could be useful when analysing the role of (elements of) a plant's architecture on its insect inhabitants.

In a study aimed at explaining the great differences in damage levels caused by noctuid larvae among individual plants of spurge laurel (*Daphne laureola*), the incidence of larvae appeared to be correlated positively with the number of leaf whorls and negatively with the mean basal diameter of stems. The uneven distribution of noctuid larvae must be attributed to their ovipositing mothers discriminating between plants on the basis of architectural features.[2]

A number of other studies have pointed to particular architectural traits affecting herbivores through effects on their natural enemies. For instance, ladybird beetle larvae captured 2.5 times more aphids on Indian ricegrass (*Oryzopsis hymenoides*) than on crested wheatgrass (*Agropyron desertorum*), two natural hosts with similar whole-plant architectures, but divergent leaf architectures.[19] Another predator, green lacewing larvae, also caught more than twice the number of aphids on Indian ricegrass. The lower predation rates on crested wheatgrass result from the aphid's preference to feed in relatively concealed locations, such as those provided by the blade–sheath junctions of mature leaves, which in this host plant are larger than in Indian ricegrass.[20] In a similar vein, the searching behaviour of insect parasitoids is often markedly affected by architectural features of the plants on which their hosts live. Thus, the attack rate of a hymenopteran parasitoid of the citrus mealybug was found to be negatively correlated with several plant characteristics, such as size, height, leaf number, leaf surface area, and branch number.[21]

When the influence of natural enemies on herbivores on two different host plant species or on different lines of the same host species is compared, it may be difficult to isolate the role of plant architecture from other (unknown) factors in which the two hosts differ. An unambiguous effect of host-plant architecture on predator efficacy could be observed in the case of ladybirds feeding on pea aphids (*Acyrthosiphon pisum*) infesting two pea (*Pisum sativum*) lines that differed at only two loci. These near-isogenic lines differ in the presence or absence of leaves. This architectural variation of pea appeared to exert dramatic effects on population dynamics of the herbivore due to differences in predation rates.[51] These few examples illustrate that host-plant architecture, through its influence on natural enemies, may be a significant factor in the population dynamics of a herbivore.

There is a clear tendency for larger and structurally more complex plants to show higher insect species richness. This is a logical consequence of the facts that (1) most herbivorous insects exploit very restricted parts of their hosts and (2) the range of ecological niches is correlated with plant complexity. Thus, the series from monocots, through herbs, to bushes and trees, which is one of increasing size and architectural complexity, is correlated with an increase in the diversity of the associated insect fauna.[59,90] Trees and bushes in Finland host more than 10 times as many macrolepidopteran species as herbs and grasses.[69]

The notion that taller plants generally house larger numbers of insects than their smaller conspecifics is exemplified by *Calluna vulgaris* vegetations, which show a striking increase in herbivore species richness and abundance with increase in vegetation height. Measurements of *Calluna* vegetation height at different localities in England and Scotland showed height levels varying between about 5 and 55 cm. The density of lepidopterous larvae approximately doubled with each 20-cm increment of *Calluna* vegetation height, partly because tall *Calluna* stands contained some species that did not occur in short stands.[40] At a quite different stratification scale the same phenomenon is found in a tropical wet forest tree, *Pourouma bicolor*. A comparison of the entomofauna of saplings (<4 m tall) with mature trees (17–30 m tall) also shows spectacular differences between herbivore abundance and species richness (Fig. 3.15).[8] Of course, the difference between conspecific plants of different ages relates to more than size alone. Several other architectural traits as well as physiological factors also change with age and it is often difficult to tell which of them produce a change in density of a particular herbivore.

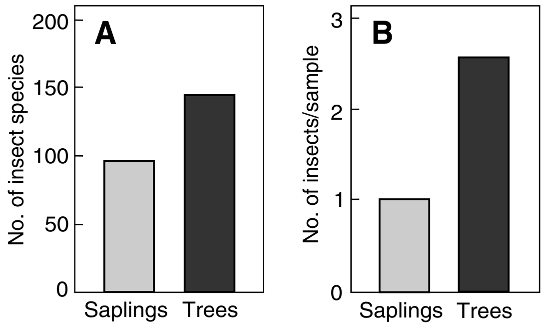


Figure 3.15 (**A**) A comparison of insect diversity (number of herbivorous insect species) and (**B**) insect abundance (average number of herbivorous insects per sample) on young and mature trees of *Pourouma bicolor*. Figures are based on a survey of 2000 leaf samples each covering 0.36 m^2 of leaf surface. (Data from Basset, 2001.)[8]

A study that identified five different architectural traits affecting herbivore diversity was made on 28 different *Opuntia* species.[67] Plant height appeared to be a key factor, as it was significantly correlated with the number of cactophilous insect species ($r = 0.59$). An alternative measure of size, namely cladode number, was an even more important plant characteristic ($r = 0.73$). When three additional variables were taken into account the correlation between plant architecture and herbivore diversity reached a still higher value ($r = 0.83$). Hence, size alone does not seem to be the only important variable (Fig. 3.16).

3.7 Conclusions

Although during the past decades more studies on interactions between plants and insect herbivores have focused on chemical factors than on the role of physical plant characteristics, there is persuasive evidence for the view that physical aspects, such as toughness and fibre content, form a stronger barrier to herbivore damage than do chemical

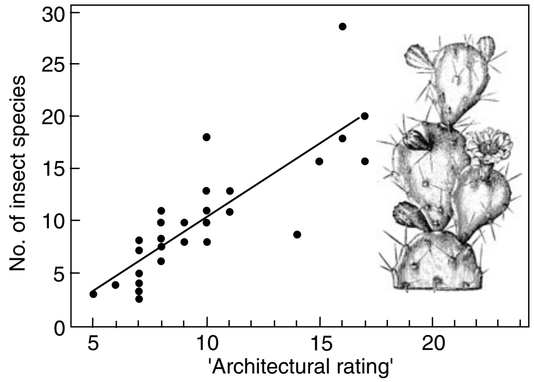


Figure 3.16 The number of herbivorous insect species associated with 28 North and South American *Opuntia* species as a function of the plants' architecture. 'Architectural rating' is the sum of the following variables scored from 1 to 4: (1) height of mature plant; (2) mean number of cladodes; (3) cladode size (cm^2); (4) development of woody stem; (5) cladode complexity (quality of cladode surface, and presence and density of spines). (From Moran, 1980.)[67]

defences.[26,83] This notion has been concluded not only from relationships of individual insect taxa with their hosts, but also from studies of the densities of herbivorous insect guilds in particular vegetations. Thus, an analysis of the functional composition of the herbivore community in an open eucalypt forest showed that the herbivore assemblage was more strongly correlated with leaf structural traits than with leaf constituents.[78]

Improvement of pest resistance in crop plants by classical breeding methods or genetic engineering requires knowledge of more precise details of herbivore feeding behaviour. To this end studies quantifying the relative importance of physical and chemical plant defences will provide essential information.[50] Special attention to the toughness of plant tissues as an efficient defence mechanism against insect herbivory is also important in view of the fact that such traits have often been eliminated during breeding of improved cultivars, especially in crop plants consumed as leaves or fruits.[76]

Clearly plant morphological aspects affect a plant's herbivores not only directly, but also indirectly, via effects on natural enemies of the herbivore. This aspect received rather scant attention until recently, although it is a promising area of research.

Many cases of morphological factors involved in insect resistance in plants have been reviewed by Norris and Kogan[70] and Panda and Khush.[76]

3.8 References

1. Agrawal, A.A. and Karban, R. (1997). Domatia mediate plant–arthropod mutualism. *Nature*, **387**, 562–63.
2. Alonso, C. and Herrera, C.M. (1996). Variation in herbivory within and among plants of *Daphne laureola* (Thymelaeaceae): correlation with plant size and architecture. *Journal of Ecology*, **84**, 495–502.
3. Bagatto, G., Paquette, L.C., and Shorthouse, J.D. (1996). Influence of galls of *Phanacis taraxaci* on carbon partitioning within common dandelion, *Taraxacum officinale*. *Entomologia Experimentalis et Applicata*, **79**, 111–17.
4. Barbehenn, R.V. (1992). Digestion of uncrushed leaf tissues by leaf-snipping larval Lepidoptera. *Oecologia*, **89**, 229–35.
5. Barbehenn, R.V. and Bernays, E.A. (1992). Relative nutritional quality of C_3 and C_4 grasses for a graminivorous lepidopteran, *Paratrytone melane* (Hesperiidae). *Oecologia*, **92**, 97–103.
6. Barbehenn, R.V., Karowe, D.N., and Chen, Z. (2004). Performance of a generalist grasshopper on a C_3 and a C_4 grass: compensation for the effects of elevated CO_2 on plant nutritional quality. *Oecologia*, **140**, 96–103.
7. Barthlott, W., Neinhuis, C., Cutler, D., Ditsch, F., Muesel, I., Theisen, I., *et al.* (1998). Classification and terminology of plant epicuticular waxes. *Botanical Journal of the Linnean Society*, **126**, 237–60.
8. Basset, Y. (2001). Communities of insect herbivores foraging on saplings versus mature trees of *Pourouma bicolor* (Cecropiaceae) in Panama. *Oecologia*, **129**, 253–60.
9. Berdegue, M. and Trumble, J.T. (1996). Effects of plant chemical extracts and physical characteristics of *Apium graveolens* and *Chenopodium murale* on host choice by *Spodoptera exigua* larvae. *Entomologia Experimentalis et Applicata*, **78**, 253–62.
10. Bergvinson, D.J., Arnason, J.T., Hamilton, R.I., Milton, J.A., and Jewel, D.C. (1994). Determining leaf toughness and its role in maize resistance to the European corn borer (Lepiodoptera: Pyralidae). *Journal of Economic Entomology*, **87**, 1743–8.
11. Bernays, E.A. (1986). Diet-induced head allometry among foliage chewing insects and its importance for graminivores. *Science*, **231**, 495–7.
12. Bernays, E.A. (1991). Evolution of insect morphology in relation to plants. *Philosophical Transactions of the Royal Society, London*, **B333**, 257–64.

13. Bernays, E.A. and Janzen, D.H. (1988). Saturniid and sphingid caterpillars: two ways to eat leaves. *Ecology*, **69**, 1153–60.
14. Brennan, E.B. and Weinbaum, S.A. (2001). Stylet penetration and survival of three psyllid species on adult leaves and 'waxy' and 'de-waxed' juvenile leaves of *Eucalyptus globulus*. *Entomologia Experimentalis et Applicata*, **100**, 355–66.
15. Burkhardt, B. and Zwölfer, H. (2002). Macroevolutionary trade-offs in the tephritid genus *Urophora*: benefits and costs of an improved plant gall. *Evolutionary Ecology Research*, **4**, 61–77.
16. Chang, G.C., Rutledge, C.E., Biggam, R.C., and Eigenbrode, S.D. (2004). Arthropod diversity in peas with normal or reduced waxy bloom. *Journal of Insect Science*, **4**(18), 1–11.
17. Chapman, R.F. (1995). Mechanics of food handling by chewing insects. In *Regulatory mechanisms in insect feeding*, (ed. R.F. Chapman and G. de Boer), pp. 3–31. Chapman & Hall, New York.
18. Chiang, H.-S. and Norris, D.M. (1983). Morphological and physiological parameters of soy bean resistance to Agromyzid bean flies. *Environmental Entomology*, **12**, 260–5.
19. Clark, T.L. and Messina, F.J. (1998a). Plant architecture and the foraging success of ladybird beetles attacking the Russian wheat aphid. *Entomologia Experimentalis et Applicata*, **86**, 153–61.
20. Clark, T.L. and Messina, F.J. (1998b). Foraging behavior of lacewing larvae (Neuroptera: Chrysopidae) on plants with divergent architectures. *Journal of Insect Behavior*, **11**, 303–17.
21. Cloyd, R.A. and Sadof, C.S. (2000). Effects of plant architecture on the attack rate of *Leptomastix dactylopii* (Hymenoptera: Encyrtidae), a parasitoid of the citrus mealybug (Homoptera: Pseudococcidae). *Environmental Entomology*, **29**, 535–41.
22. Coley, P.D. (1983). Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs*, **53**, 209–33.
23. Dalin, P. and Björkman, C. (2003). Adult beetle grazing induces willow trichome defence against subsequent larval feeding. *Oecologia*, **134**, 112–18, 554.
24. Davies, R.G. (1988). *Outlines of entomology* (7th edn). Chapman & Hall, London.
25. Dawkins, R. (1982). *The extended phenotype*. Oxford University Press, Oxford.
26. Dyer, L.A. and Coley, P.D. (2002). Tritrophic interactions in tropical versus temperate communities. In *Multitrophic level interactions* (ed. T. Tscharntke and B.A. Hawkins), pp. 67–88. Cambridge University Press, Cambridge.
27. Eigenbrode, S.D. (2004). The effects of plant epicuticular waxy blooms on attachment and effectiveness of predatory insects. *Arthropod Structure and Development*, **33**, 91–102.
28. Eigenbrode, S.D. and Espelie, K.E. (1995). Effects of plant epicuticular lipids on insect herbivores. *Annual Review of Entomology*, **40**, 171–94.
29. Eigenbrode, S.D., Kabalo, N.N., and Stoner, K.A. (1999). Predation, behavior, and attachment by *Chrysoperla plarabunda* larvae on *Brassica oleracea* with different surface waxblooms. *Entomologia Experimentalis et Applicata*, **90**, 225–35.
30. English-Loeb, G., Norton, A.P., and Walker, M.A. (2002). Behavioral and populations consequences of acarodomatia in grapes on phytoseiid mites (Mesostigmata) and implications for plant breeding. *Entomologia Experimentalis et Applicata*, **104**, 307–19.
31. Epstein, E. (1999). Silicon. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**, 641–64.
32. Fernandes, G.W. and Negreiros, D. (2001). The occurrence and effectiveness of hypersensitive reaction against galling herbivores across host taxa. *Ecological Entomology*, **26**, 46–55.
33. Frost, S.W. (1959). *Insect life and insect natural history* (2nd edn). Dover Publications, New York.
34. Gartner, B.L. (2001). Multitasking and tradeoffs in stems, and the costly dominion of domatia. *New Phytologist*, **151**, 311–13.
35. Godin, C., Costes, E., and Sinoquet, H. (1999). A method for describing plant architecture which integrates topology and geometry. *Annals of Botany*, **84**, 343–57.
36. Gorb, E.V. and Gorb, S.N. (2002). Attachment ability of the beetle *Chrysolina fastuosa* on various plant surfaces. *Entomologia Experimentalis et Applicata*, **105**, 13–28.
37. Grostal, P. and O'Dowd, D.J. (1994). Plants, mites and mutulism: leaf domatia and the abundance and reproduction of mites on *Viburnum tinus* (Caprifoliaceae). *Oecologia*, **97**, 308–15.
38. Hanan, J., Prusinkiewicz, P., Zalucki, M., and Skirvin, D. (2002). Simulation of insect movement with respect to plant architecture and morphogenesis. *Computers and Electronics in Agriculture*, **35**, 255–69.
39. Harris, K.F., Smith, O.P., and Duffus, J.E. (ed.) (2001). *Virus–insect–plant interactions*. Academic Press, San Diego.
40. Haysom, K.A. and Coulson, J.C. (1999). The Lepidoptera fauna associated with *Calluna vulgaris*: effects of plant architecture on abundance and diversity. *Ecological Entomology*, **23**, 377–85.
41. Heming, B.S. (1993). Structure, function, ontogeny, and evolution of feeding in thrips (Thysanoptera). In

Functional morphology of insect feeding (ed. C.W. Schaefer and R.A.B. Leschen), pp. 5–41. Entomological Society of America, Lanham.

42. Hilker, M., Rohfritsch, O., and Meiners, T. (2002). The plant's response towards insect egg deposition. In *Chemoecology of insect eggs and egg deposition* (ed. M. Hilker and T. Meiners), pp. 205–33. Blackwell, Berlin.
43. Hochuli, D.F. (1996). The ecology of plant/insect interactions: implications of digestive strategy for feeding by phytophagous insects. *Oikos*, **75**, 133–41.
44. Hochuli, D.F. and Roberts, F.M. (1996). Approximate digestibility of fibre for a graminivorous caterpillar. *Entomologia Experimentalis et Applicata*, **81**, 15–20.
45. Howlett, B.G., Clarke, A.R., and Madden, J.L. (2001). The influence of leaf age on the oviposition preference of *Chrysophtharta bimaculata* (Olivier) and the establishment of neonates. *Agricultural and Forest Entomology*, **3**, 121–27.
46. Huxley, C.R. and Cutler, D.F. (ed.) (1991). *Ant–plant interactions*. Oxford University Press, Oxford.
47. Isely, F.B. (1944). Correlation between mandibular morphology and food specificity in grasshoppers. *Annals of the Entomological Society of America*, **37**, 47–67.
48. Jeffree, C.E. (1986). The cuticle, epicuticular waxes and trichomes of plants, with reference to their structure, functions and evolution. In *Insects and the plant surface*, (ed. B.E. Juniper and T.R.E. Southwood), pp. 23–64. E. Arnold, London.
49. Jetter, R., Schäffer, S., and Riederer, M. (2000). Leaf cuticular waxes are arranged in chemically and mechanically distinct layers: evidence from *Prunus laurocerasus* L. *Plant and Environment*, **23**, 619–28.
50. Kanno, H. and Harris, M.O. (2000). Leaf physical and chemical features influence selection of plant genotypes by Hessian fly. *Journal of Chemical Ecology*, **26**, 2335–54.
51. Kareiva, P. and Sahakian, R. (1990). Tritrophic efects of a simple architectural mutation in pea plants. *Nature*, **345**, 433–4.
52. Keeping, M.G. and Meyer, J.H. (2002). Calcium silicate enhances resistance of sugarcane to the African stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Agricultural and Forest Entomology*, **4**, 265–74.
53. Kennedy, C.E.J. (1986). Attachment may be a basis for specialization in oak aphids. *Ecological Entomology*, **11**, 291–300.
54. Kennedy, C.E.J. and Southwood, T.R.E. (1984). The number of species of insects associated with British trees: a re-analysis. *Journal of Animal Ecology*, **53**, 455–78.
55. Khan, Z.R. and Ramachandran, R. (1989). Studies on the biology of the yellow stem borer, *Scirpophaga incertulas*. *ICIPE Annual Report*, 1989, 24–5.
56. Kingsolver, J.G. and Daniel, T.L. (1995). Mechanics of food handling by fluid-feeding insects. In: *Regulatory mechanisms in insect feeding* (ed. R.F. Chapman and G. de Boer), pp. 32–73. Chapman & Hall, New York.
57. Koyama, Y., Yao, I., and Akimoto, S.-I. (2004). Aphid galls accumulate high concentrations of amino acids: a support for the nutrition hypothesis for gall formation. *Entomologia Experimentalis et Applicata*, **113**, 35–44.
58. Labandeira, C.C. (1997). Insect mouthparts: ascertaining the paleobiology of insect feeding strategies. *Annual Review of Ecology and Systematics*, **28**, 153–93.
59. Lawton, J.H. (1983). Plant architecture and the diversity of phytophagous insects. *Annual Review of Entomology*, **28**, 23–39.
60. Lee, Y.L., Kogan, M., and Larsen, J.R. (1986). Attachment of the potato leafhopper to soybean plant surfaces as affected by morphology of the pretarsus. *Entomologia Experimentalis et Applicata*, **42**, 101–8.
61. Merz, E. (1959). Pflanzen und Raupen. Über einige Prinzipien der Futterwahl bei Großschmetterlingsraupren. *Biologisches Zentralblatt*, **78**, 152–88.
62. Meyer, G.A. (1993). A comparison of the impacts of leaf- and sap-feeding insects on growth and allocation of goldenrod. *Ecology*, **74**, 1101–16.
63. Miles, P.W. (1999). Aphid saliva. *Biological Reviews*, **74**, 41–85.
64. Miller, W.E. (2004). Host breadth and voltinism in gall-inducing Lepidoptera. *Journal of the Lepidopterists' Society*, **58**, 44–7.
65. Moore, D. (1984). The role of silica in protecting Italian ryegrass (*Lolium multiflorum*) from attack by dipterous stem-boring larvae (*Oscinella frit* and other related species). *Annals of Applied Biology*, **104**, 161–6.
66. Moraes, J.C., Goussain, M.M., Basagli, M.A.B., Carvalho, G.A., Ecole, C.C., and Sampaio, M.V. (2004). Silicon influence on the tritrophic interaction: wheat plants, the greenbug *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), and its natural enemies, *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) and *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae). *Neotropical Entomology*, **33**, 619–24.
67. Moran, V.C. (1980). Interactions between phytophagous insects and their *Opuntia* hosts. *Ecological Entomology*, **5**, 153–64.
68. Mullin, C.A. (1986). Adaptive divergence of chewing and sucking arthropods to plant allelochemicals. In: *Molecular aspects of insect–plant associations* (ed. L.B. Brattsten and S. Ahmad), pp. 175–209. Plenum Press, New York.

69. Niemelä, P., Tahvanainen, J., Sorjonen, J., Hokkanen, T., and Neuvonen, S. (1982). The influence of host plant growth form and phenology on the life strategies of Finnish macrolepidopterous larvae. *Oikos*, **39**, 164–70.
70. Norris, D.M. and Kogan, M. (1980). Biochemical and morphological bases of resistance. In *Breeding plants resistant to insects* (ed. F.G. Maxwell and P.R. Jennings), pp. 23–61. Wiley, New York.
71. Norton, A.P., English-Loeb, G., and Belden, E. (2001). Host plant manipulation of natural enemies: leaf domatia protect beneficial mites from predators. *Oecologia*, **126**, 535–42.
72. Nyman, T. and Julkunen-Tiitto, R. (2000). Manipulation of the phenolic chemistry of willows by gall-inducing sawflies. *Proceedings of the National Academy of Sciences of the USA*, **97**, 13184–7.
73. O'Dowd, D.J. and Pemberton, R.W. (1998). Leaf domatia and foliar mite abundance in broadleaf deciduous forest of north Asia. *American Journal of Botany*, **85**, 70–8.
74. O'Dowd, D.J. and Willson, M.F. (1991). Associations between mites and leaf domatia. *Trends in Evolotion and Ecology*, **6**, 179–82.
75. Pagani, M., Freeman, K.H., and Arthur, M.A. (1999). Late Miocene atmospheric CO_2 concentrations and the expansion of C_4 grasses. *Science*, **285**, 876–9.
76. Panda, N. and Khush, G.S. (1995). *Host plant resistance to insects*. CAB International, London.
77. Pappers, S.M., Van Dommelen, H., Van der Velde, G., and Ouborg, N.J. (2001). Differences in morphology and reproductive traits of *Galerucella nymphaeae* from four host plant species. *Entomologia Experimentalis et Applicata*, **99**, 183–91.
78. Peeters, P.J. (2002). Correlations between leaf structural traits and the densities of herbivorous insect guilds. *Biological Journal of the Linnean Society*, **77**, 43–65.
79. Plumb, R.T. (ed.) (2002). Plant virus vector interactions. *Advances in Botanical Research*, **36**, 1–221.
80. Raven, J.A. (1983). Phytophages of xylem and phloem. *Advances in Ecological Research*, **13**, 135–234.
81. Redfern, M. (1997). Plant galls: an intimate association between animals and plants. *Antenna*, **21**, 55–63.
82. Robinson, S.J., Thurston, R., and Jones, G.A. (1980). Antixenosis of smooth leaf cotton *Gossypium* spp. to the ovipositional response of the tobacco budworm *Heliothis virescens*. *Crop Science*, **20**, 646–49.
83. Sanson, G., Read, J., Aranwela, N., Clissold, F., and Peeters, P. (2001). Measurement of leaf biomechanical properties in studies of herbivory: opportunities, problems and procedures. *Austral Ecology*, **26**, 535–46.
84. Scheirs, J., de Bruyn, L., and Verhagen, R. (2001). A test of the C_3–C_4 hypothesis with two grass miners. *Ecology*, **82**, 410–21.
85. Schofield, R.M.S., Nesson, M.H., and Richardson, K.A. (2002). Tooth hardness increases with zinc-content in mandibles of young adult leaf-cutter ants. *Naturwissenschaften*, **89**, 579–83.
86. Schoonhoven, L.M. and Henstra, S. (1972). Morphology of some rostrum receptors in *Dysdercus* spp. *Netherlands Journal of Zoology*, **22**, 343–46.
87. Shorthouse, J.D. and Rohfritsch, O. (eds) (1992). *Biology of insect-induced galls*. Oxford University Press, New York.
88. Southwood, T.R.E. (1986). Plant surfaces and insects—an overview. In *Insects and the plant surface* (ed. B. Juniper and T.R.E. Southwood), pp. 1–22. E. Arnold, London.
89. Stone, G.N. and Schönrogge, K. (2003). The adaptive significance of insect gall morphology. *Trends in Ecology and Evolution*, **18**, 512–22.
90. Strong, D.R., Lawton, J.H., and Southwood, T.R.E. (1984). *Insects on plants. Community patterns and mechanisms*. Blackwell, Oxford.
91. Tjallingii, W.F. (1978). Mechanoreceptors of the aphid labium. *Entomologia Experimentalis et Applicata*, **24**, 731–7.
92. Tjallingii, W.F. (1995). Regulation of phloem sap feeding by aphids. In *Regulatory mechanisms in insect feeding* (ed. R.F. Chapman and G. de Boer), pp. 190–209. Chapman & Hall, New York.
93. Traw, M.B. and Dawson, T.E. (2002). Differential induction of trichomes by three herbivores of black mustard. *Oecologia*, **131**, 526–32.
94. Van Lenteren, J.C., Hua, L.Z, Kamerman, J.W., and Xu, R. (1995). The parasite–host relationship between *Encarsia formosa* (Hym., Aphelinidae). and *Trialeurodes vaporariorum* (Hom., Aleyrodidae). XXVI. Leaf hairs reduce the capacity of *Encarsia* to control greenhouse whitefly on cucumber. *Journal of Applied Entomology*, **119**, 553–9.
95. Werker, E. (2000). Trichome diversity and development. *Advances in Botanical Research*, **31**, 1–36.
96. White, C. and Eigenbrode, S.D. (2000). Leaf surface waxbloom in *Pisum sativum* influences predation and intra-guild interactions involving two predator species. *Oecologia*, **124**, 252–59.
97. Williams, M.A.J. (ed.) (1994). *Plant galls: organisms, interactions, populations*. Clarendon, Oxford.

CHAPTER 4

Plant chemistry: endless variety

On the face of it, plants, which cannot fight or flee and which often have long generation spans and low recombination rates, appear to be at a disadvantage when compared to the herbivores consuming them. Insects, especially, can often adapt rapidly to changing conditions, because of their small size and concomitant relatively short generation span, combined with a high reproductive capacity. Moreover, insects profit from being winged, as this permits them to disperse and invade potential food sources even at considerable distances from their place of birth and larval domicile. Despite the apparent vulnerability of plants to herbivore attack, the Earth's flora has evolved to a green and highly diverse blanket. Plants clearly possess an effective resistance system, based on a combination of physical, chemical, and developmental features. The term *resistance* is (in the context of insect–plant interactions) used to describe a plant's capacity to avoid or reduce damage to herbivory. It is not synonymous with *defence*, because the latter term implies something about the evolutionary *raison d'être* for the trait, and indicates that the resistance trait has evolved or is maintained in the plant population because of selection exerted by herbivores or other natural enemies. The term resistance is of an empirical nature and is commonly used in the applied literature (see Chapter 13). Its use is preferable in those cases for which assumptions on defensive functions of plant traits are (still) unproved.[151,213]

Chemical characteristics of plants have attracted the interests of many students of insect–plant relationships, resulting in a large and flourishing literature on the subject. It is now recognized that the plant world is characterized by a bewildering proliferation of secondary metabolites. More than 80% of the presently known natural compounds have a botanical origin.[119] As chemicals produced by plants play, in addition to physical barriers, a cardinal role in controlling insect behaviour in nature, much of this chapter deals with a description of the nature and dynamics of secondary plant substances. An elementary knowledge of phytochemistry is essential to comprehend insect–plant interactions fully.

Entomologists, although well aware of variations in the morphology and behaviour of insects, may envision plants as a homogeneous resource for herbivores and consider, by and large, that the chemical composition of a specific plant part, for instance a leaf, within each plant and between individual plants is similar. This is a misconception. In this chapter the view is developed that plants are highly heterogeneous hosts in space and time.

There is increasing evidence to support the idea that heterogeneity in chemical and structural composition, together with interplant variation, is crucial for preventing herbivorous insects from fully exploiting their host plants. Insects, often highly specialized and adapted to certain diets only, face decreased fitness via both direct and indirect pathways on resources of variable composition.[65]

4.1 Plant biochemistry

Plants share with all other living organisms a number of biochemical reactions that maintain their basic or primary metabolism, which is involved in the formation and breakdown of a limited set of chemicals. These include nucleic acids and proteins with their precursors, particular carbohydrates, carboxylic acids, etc.[38] Based on this primary metabolism, plants have evolved a corona of secondary metabolic pathways producing an extraordinary array of secondary plant substances. The large variety of secondary constituents is produced via only three main biogenetic routes, each leading to one or a few key metabolites, from which numerous derivatives are formed, usually by a consecutive series of enzymatic transformations.[125] To date, few biosynthetic routes of secondary compounds have been fully elucidated. Often, they are very complex, as in the case of the synthesis of taxol, a strong insect-feeding deterrent that is found in the leaves and bark of yew trees.[61] Its manufacture involves 20 enzymatic conversions.[283]

It should be emphasized that, although the adjectives 'primary' and 'secondary' might suggest a sharp distinction between both metabolic systems, this is not the case. The sugar alcohol sorbitol (**64**), for instance, which is rarely found outside the ligneous Rosaceae, functions in hawthorn (*Crataegus monogyna*), apple (*Malus domestica*), and other congeneric species as the major soluble carbohydrate. (Bold numbers refer to the molecular structures in Appendix B.) At leaf concentrations of up to 11% of dry weight, sorbitol serves as the primary energy carrier,[99,159] and in this case it seems difficult to attach the label 'secondary' to this compound. Moreover, primary and secondary metabolism are strongly intertwined and the division that has been made between primary and secondary plant substances is therefore arbitrary and for convenience only. In nature, the two systems operate in concert.[23]

4.1.1 Primary plant metabolism

Photosynthesis is the process by which green plants capture solar energy and store this into sugars, the most basic chemical energy source. Part of this energy is used to convert nitrogen to amino acids, the building blocks of proteins. Sugars are also incorporated in the structural elements of cell walls. The greater part of a plant's biomass consists of primary plant substances. Some of them occur in great quantities; lignocellulose, for instance, is the most abundant organic polymer on earth. Cellulose and hemicellulose (both complex polysaccharides) and lignin (a phenolic polymer) together constitute (by dry mass) approximately 90% of deciduous wood, 66% of grass, and 50% of deciduous leaves.[2] The main groups of primary plant metabolites—proteins, carbohydrates, and lipids involved in fundamental plant physiological processes—form essential nutrients for herbivores. Therefore, qualitative and quantitative variation in primary plant compounds can have profound effects on insect preference and performance.[22] This aspect is discussed in Chapter 5.

4.1.2 Secondary plant substances

Secondary plant substances can be defined as 'plant compounds that are not universally found in higher plants, but are restricted to certain plant taxa, or occur in certain plant taxa at much higher concentrations than in others, and have no (apparent) role in primary metabolism.' Although chemical differences between plant species have been recognized since ancient times, notions of their function developed gradually only during the twentieth century. In a seminal paper Fraenkel stressed the role of secondary plant substances as a defence system against insects and other natural enemies.[95] Although there is undoubtedly much compelling evidence for that supposition, critics of this one-sided concept have emphasized that many secondary plant substances appear to have other (additional) functions within the plant. They argue that the defensive role of these compounds may simply be pleiotropic effects of genes controlling resistance factors that were selected in response to other environmental stresses. Thus, competition between conspecific and heterospecific plants (allelopathy[43]), nutrient deficiency (e.g. alkaloids as nitrogen reserves), drought, and ultraviolet radiation have been suggested as environmental factors that have stimulated the evolution of the vast biochemical machinery serving the production of secondary plant substances. This point is discussed in more detail in Chapter 11.

Because of their ecological role, secondary plant substances can be classified as 'allelochemics', a term coined by Whittaker. An allelochemic is defined as a 'non-nutritional chemical produced by an individual of one species that affects the growth, health, behaviour, or population biology of another species'.[290] Contrary to what the adjective suggests, secondary plant substances play a primary ecological role in plants. In contrast to the relative monotony of their primary metabolic profiles, plants produce an astonishing array of secondary metabolites (Fig. 4.1). Even one single plant species may produce an extensive pharmacopeia of recondite chemicals. Periwinkle (*Catharanthus roseus*), for instance, contains more than 100 different monoterpenoid indole alkaloids,[38] and the berries of grapevine (*Vitis vinifera*) accumulate more than 200 different aglycones conjugated to glucose.[237] Because of the large number of secondary compounds in a plant species, and the many enzymatic steps involved in their production, it must be assumed that the number of metabolites found in one species exceeds the number of genes involved in their biosynthesis.[235]

It has been estimated that the plant kingdom synthesizes hundreds of thousands of different secondary plant substances. The number of identified compounds now exceeds 100 000 and new structures are reported daily in the scientific literature.[29,235] Clearly the chemical world in which a plant-feeding insect finds itself is exceedingly complex.

It is difficult to construct a satisfactory classification of secondary plant substances, for instance based on molecular structures. As secondary metabolites are produced from universally present precursors, most often acetyl-coenzyme A, amino

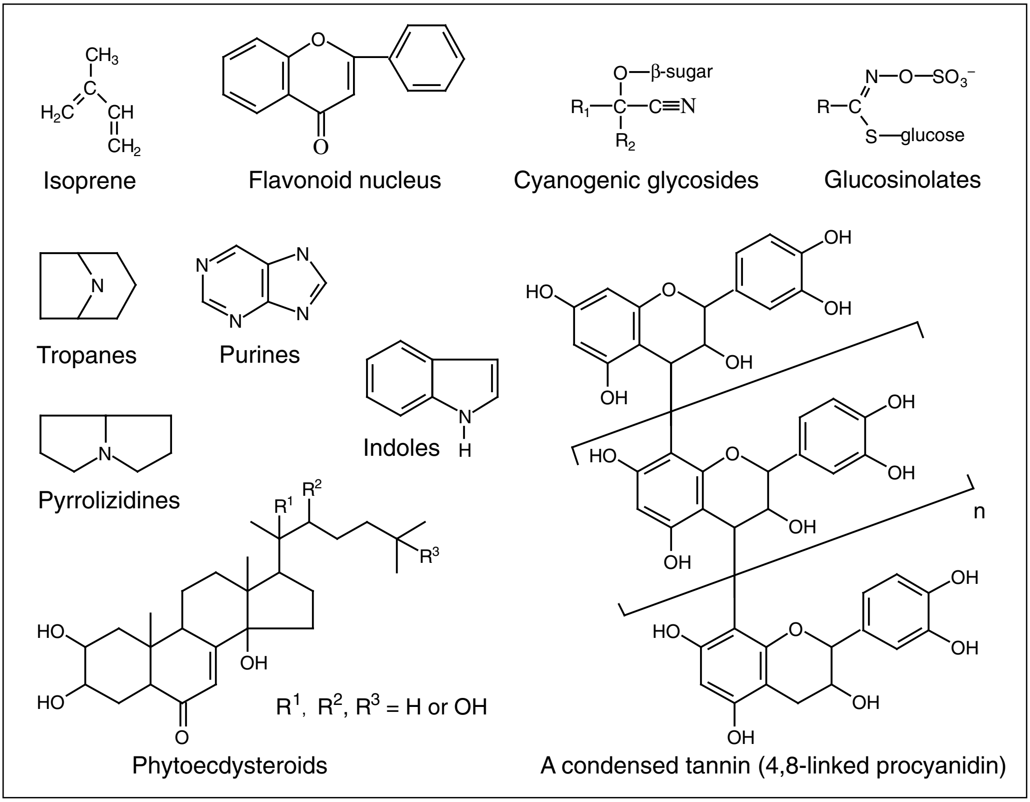


Figure 4.1 General molecular structures of major groups of secondary plant substances.

acids, or shikimate, a classification derived from their biosynthetic pathways (Fig. 4.2) appears to be suitable for most cases.[38,176,223]

Starting from these few basic chemicals, the synthesis of secondary compounds often involves 'polydimensional networks'—different pathways interconnected in several places. A simplified classification distinguishes: (1) nitrogen-containing compounds, (2) terpenoids, (3) phenolic compounds, and (4) acetylenic compounds (Table 4.1).

4.2 Alkaloids

Alkaloids are cyclic nitrogen-containing compounds with a limited distribution among living organisms. They include a vast array of chemicals that are often structurally unrelated. Alkaloids are often distinguished on the basis of their precursor molecules. Most of them seem to be derived from a fairly restricted range of common amino acids, such as lysine, tyrosine, tryptophan, histidine, and ornithine.[89] Nicotine (**41**), for instance, is produced from ornithine and nicotinic acid. Among the best known representatives of the *benzyl isoquinoline alkaloids* are papaverine (**42**), berberine (**7**), and morphine. Most of the curare alkaloids also fall into this group, including tubocurarine (**70**). Many of the alkaloids particularly characteristic of the Solanaceae belong to the *tropane alkaloids*. Atropine (**5**), found in deadly nightshade (*Atropa belladonna*), and scopolamine (**57**) serve as examples. Cocaine (**13**) and related alkaloids from the coca plant (*Erythroxylon coca*) are of the same type, but do not occur in the Solanaceae. The so-called *indole alkaloids* owe their name to the presence of an indole nucleus. Two well known compounds, strychnine (**65**) and quinine (**50**), which have a bitter taste to us and are strongly deterrent to many

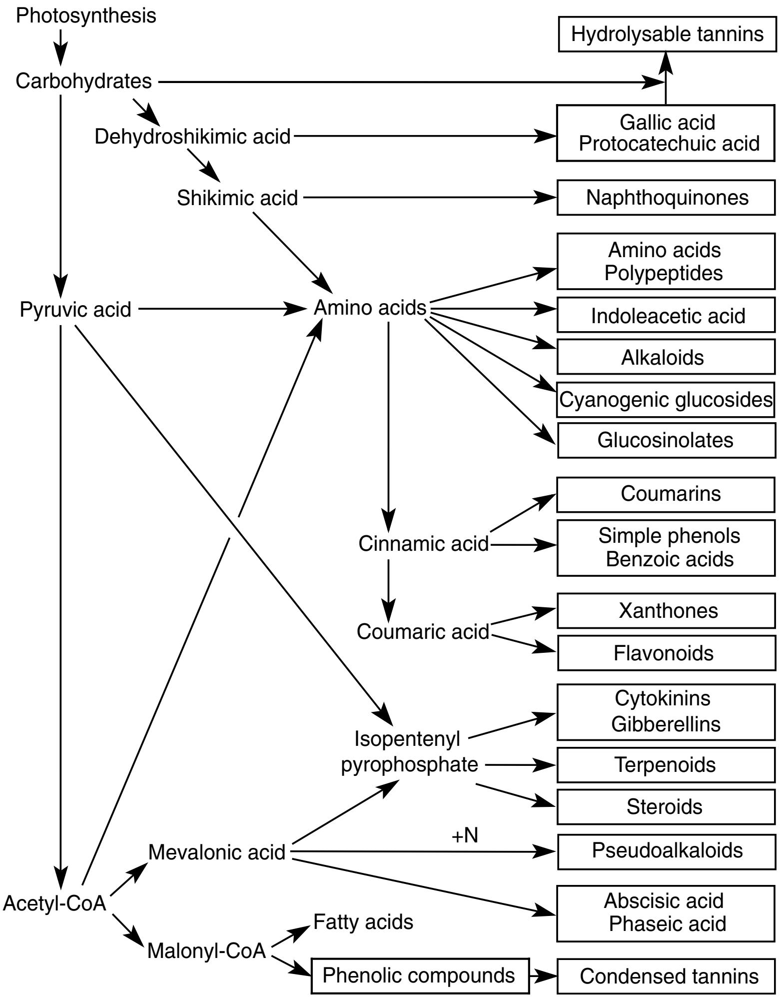


Figure 4.2 Biosynthetic routes of major groups of secondary plant substances.

insects, belong to this group of alkaloids. *Pyrrolizidine alkaloids* (PAs) are ester alkaloids. Their biosynthesis has been studied most extensively in *Senecio* species. Senecionine (**59**) is a noxious macrocyclic diester. The *quinolizidine alkaloids*, which are derived from lysine, are frequently called lupin alkaloids, because of their general abundance in the genus *Lupinus*. *Polyhydroxy alkaloids* have recently been recognized as compounds that stereochemically mimic sugars, thereby interfering with glycosidases. They act as feeding deterrents against various insects.[92] Some other alkaloids are derived from nicotinic acid, purines, anthranilic acid, polyacetates, and terpenes. They include the *purine alkaloids*, for example caffeine (**9**).[13]

About 20% of the angiosperms produce alkaloids. Alkaloids are rarely found in gymnosperms (e.g. conifers) or cryptogams (e.g. ferns). Most alkaloids act as feeding deterrents and/or toxins to most insect species at dietary concentrations over 0.1% w/w.

4.3 Terpenoids and steroids

Terpenoids are the largest group of secondary compounds (roughly 30 000 currently fully characterized). This group shows an incredible structural

Table 4.1 Major classes of secondary plant compounds with significant roles in insect–plant interactions (modified from Harborne, 1993)[119]

Class	No. of known compounds	Distribution in vascular plants	Physiological activity
Nitrogen-containing compounds			
Alkaloids	16 000	Widely in angiosperms, especially in root, leaf and fruit	Many toxic and bitter-testing;
Amines	100	Widely in angiosperms, often in flowers	Many repellent
Amino acids (non-protein)	400	Especially in seeds of legumes, but relatively widespread	Many toxic
Cyanogenic glycosides	60	Sporadic, especially in fruit and leaf	Poisonous (as HCN)
Glucosinolates	120	Brassicaceae and occasionally in 10 other families	Acrid and bitter (as isothiocyanates)
Terpenoids	30 000		
Monoterpenes	1000	Widespread in essential oils	Pleasant smells
Sesquiterpenes	6500	In Angiospermae, especially in Asteraceae, in essential oils and resins	Some bitter and toxic
Diterpenes	3000	Widespread, especially in latex and resins	Some toxic
Saponins	600	In over 70 plant families, especially Lilliflorae, Solanaceae, Scrophulariaceae	Toxic (haemolytic)
Limonoids	300	Predominantly in Rutaceae, Meliaceae	Bitter-tasting
Cucurbitacins	50	Predominantly in Cucurbitaceae	Bitter-tasting and toxic
Cardenolides	150	In 12 angiosperm families, especially in Apocynaceae and Asclepiadaceae	Toxic and bitter
Carotenoids	650	Universal in leaf, often in flower and fruit	Pigments
Other	1500	Widespread	
Phenolics			
Simple phenols	200	Universal in leaf, often also in other tissues	Antimicrobial
Flavonoids (incl. tannins)	8000	Universal in Angiospermae, Gymnospermae, and ferns	Often pigments
Quinones	800	Widespread, especially in Rhamnaceae	Pigments
Polyacetates			
Polyacetylenes	750	Mainly in Asteraceae and Apiaceae	Some toxic

The approximate numbers of known compounds reflect present knowledge, but will soon become outdated because of continuous discovery of new structures.

diversity of compounds biosynthesized through either of two pathways: the mevalonate pathway in the cytosol leads to sesquiterpenes, triterpenes, sterols, and polyterpernes, whereas the more recently discovered deoxy-D-xylulose pathway in plastids results in isoprene, monoterpenes, diterpenes, and carotenoids.[172] Most terpenoids can be viewed as being built up of isoprene units (see Fig. 4.1), linked together in various ways with different types of ring closure and varying in level of saturation and functional groups. Isoprene itself is emitted in huge amounts from many plants, especially at high temperatures.[239] Terpenoids can be classified according to the number of their constituent isoprene units (Table 4.2).

Most *monoterpenoids* are volatile compounds, found mainly as components of essential oils. They occur in a wide diversity of angiosperm and gymnosperm species, imparting a characteristic flavour and odour to the tissue in which they occur. Monoterpenoids may be acyclic (with an open ring), for example geraniol (**24**), monocyclic, for example limonene (**34**), or bicyclic, for example pinene (**46**). In order to prevent autotoxicity, monoterpenoids require specialized storage structures in the plant for sequestration.

Table 4.2 Major classes of plant terpenoids

Terpenoid category and general formula	Plant product	Principal types
Hemiterpenoids (C_5H_8)	Essential oils	Tuliposides
Monoterpenoids ($C_{10}H_{16}$)	Essential oils	Iridoids
Sesquiterpenoids ($C_{15}H_{24}$)	Essential oils, resins	Sesquiterpene lactones
Diterpenoids ($C_{20}H_{32}$)	Resins, bitter extracts	Clerodanes, tiglianes, gibberellins
Triterpenoids ($C_{30}H_{48}$)	Resins, latex, corks, cutins	Sterols, cardiac glycosides (cardenolides), phytoecdysteroids, cucurbitacins, saponins
Tetraterpenoids ($C_{40}H_{64}$)	Pigments	Carotenes, xanthophylls
Polyterpenoids [$(C_5H_8)_n$]	Latex	Rubber, balata, gutta

The largest class of terpenoids comprises the *sesquiterpenoids*, which are also commonly found in essential oils. Well known examples of the drimane-type aldehydes are polygodial (**47**) and warburganal (**74**), which act as feeding deterrents to a broad range of insect species.[266] Sesquiterpene lactones possess a five-membered lactone ring, as exemplified by glaucolide A (**26**). They occur frequently in the Asteraceae family, where they are localized in glandular hairs or in latex ducts. Gossypol (**28**) is a well known phenolic sesquiterpene dimer found in cotton (*Gossypium* sp.) and related genera of the family Malvaceae. Monoterpene and sesquiterpene hydrocarbons are relatively weak odorants for humans, but often serve as important olfactory cues to insects.

Diterpenoids include resin acids in conifers (e.g. abietic acid (**1**)) and the clerodanes, such as clerodin (**12**) from the Indian bhat tree and ajugarin (**3**) from the leaves of *Ajuga remota*. The clerodanes are potent feeding deterrents to many insect species.[42]

Triterpenoids are widespread and diverse, occurring in resins, cutins, and corks. They include the limonoids (with azadirachtin (**6**) as one of the strongest insect-feeding deterrents known[194]), the lantadenes, and the cucurbitacins (e.g. cucurbitacin B (**14**)). The latter compounds, which taste intensely bitter to humans, deter feeding in many herbivorous insects.[18,257] There are, on the other hand, also several insect species specialized on cucurbit plants that use cucurbitacins as powerful host-recognition cues.[1]

Saponins contain a polycyclic aglycone moiety of either triterpenoid (C_{30}) or steroid (C_{27}) structure attached to a sugar moiety. Aescin (**2**) and dioscin (**19**) are examples of the two types, occurring in horse chestnut (*Aesculus hippocastaneum*) and yams (*Dioscorea* spp.), respectively. Saponins have been identified in 80 plant families and many were shown to interfere with insect growth and development.[4] Insects are unable to synthesize the steroid nucleus in quantity and must obtain cholesterol or sitosterol (**62**) from their diet for the synthesis of steroid hormones such as the moulting hormone ecdysone (**21**). A number of plant species (5–6%) produce ecdysone and closely resembling derivatives, which are called *phytoecdysteroids* (see Fig. 4.1). In particular, some ferns and gymnosperms may contain concentrations up to five orders of magnitude above those occurring in insects. Rhizomes of the common fern (*Polypodium vulgare*), for instance, contain up to 1% β-ecdysone (i.e. the major insect ecdysteroid), and dry stems of *Diploclisia glaucescens* (Menispermaceae) have been reported to contain as much as 3.2% of this phytoecdysteroid. Because a true physiological role of ecdysteroids in plants is unknown, it seems attractive to postulate that they serve primarily as a defence mechanism against insect herbivores. The experimental evidence for this assumption is at the present time still meagre.[80,181]

Some compounds that are of terpenoid origin but appear to have lost or gained carbon atoms include the gibberellins (**25**), which function as hormones in higher plants, the tocopherols (e.g. vitamin E (**73**)), which act as antioxidants in seed oils, and the active principles of marihuana (e.g. cannabidiol (**10**)).

Terpenoids often occur in plants as complex mixtures. The essential oil of carrot leaves, for instance, may contain up to 30 different terpenoids[148] and 23 terpenoid components have been extracted from resin in the stems of Norway spruce trees (*Picea abies*).[182]

4.4 Phenolics

Phenolic compounds are ubiquitous in plants.[120,285] They possess an aromatic ring with one or more hydroxyl groups, together with a number of other constituents. The name of this group derives from the simple aromatic parent substance phenol (**44**), but most contain more than one hydroxyl group (polyphenols). They are conveniently classified according to the number of carbon atoms in the basic skeleton (Table 4.3).

A group of relatively simple phenolics include the hydroxybenzoic acids (e.g. vanillic acid (**72**)), the hydroxycinnamic acids (e.g. caffeic acid (**8**)), and the coumarins. Examples of the latter category are umbelliferone (**71**), widespread in the Apiaceae, and scopoletin (**58**), commonly occurring in solanaceous plants but also present in other families.

By far the largest and most diverse group of plant phenolics are the *flavonoids*, which occur universally in higher plants. Therefore, almost all herbivores encounter these secondary metabolites when feeding. Usually, a plant contains several representatives of this group of compounds and almost every plant species possesses its own distinctive flavonoid profile. The flavonoids share a basic C_6-C_3-C_6 structure (Fig. 4.1; e.g. kaempferol (**33**)). The flavonoid nucleus is normally linked to a sugar moiety to form a water-soluble glycoside. Most flavonoids are stored in the plant cell vacuoles. The flavonoids can be subdivided into flavones (e.g. luteolin (**36**)), flavanones (e.g. naringenin (**40**)), flavonols (e.g. kaempferol (**33**)), anthocyanins, and chalcones. Many flavones, flavanones, and flavonols absorb light in the visible region and hence give flowers and other plant parts their bright yellow or cream colours. Many colourless representatives of these groups are often of considerable

Table 4.3 Major classes of phenolics in plants (modified from Harborne, 1994)[120]

Basic skeleton	No. of carbon atoms	Class	Examples
C_6	6	Simple phenols	Catechol, hydroquinone
		Benzoquinones	2,6-Dimethoxybenzoquinone
C_6–C_1	7	Phenolic acids	*p*-Hydroxybenzoic, salicylic acid
C_6–C_2	8	Acetophenones	3-Acetyl-6-methoxybenzaldehyde
		Phenylacetic acids	*p*-Hydroxyphenylacetic acid
C_6–C_3	9	Hydroxycinnamic acids	Caffeic acid, ferulic acid
		Phenylpropenes	Myristicin, eugenol
		Coumarins	Umbelliferone, aesculetin
		Isocoumarins	Bergenin
		Chromones	Eugenin
C_6–C_4	10	Naphthoquinones	Juglone, plumbagin
C_6–C_1–C_6	13	Xanthones	Mangiferin
C_6–C_2–C_6	14	Stilbenes	Lunularic acid
		Anthraquinones	Emodin
C_6–C_3–C_6	15	Flavonoids	Quercetin, malvin
		Isoflavonoids	Genistein
$(C_6$–$C_3)_2$	18	Lignans	Podophyllotoxin
$(C_6$–C_3–$C_6)_2$	30	Biflavonoids	Amentoflavone
$(C_6$–$C_3)_n$	9n	Lignins	
$(C_6)_n$	6n	Catechol melanins	
$(C_6$–C_3–$C_6)_n$	15n	Flavolans (condensed tannins)	

significance as feeding deterrents (e.g. catechin) or as insect toxicants (e.g. the natural insecticide rotenone (**52**)). Phaseolin (**43**), for instance, is among the most potent feeding deterrents ever recorded. In tests with root-feeding larvae of the beetle *Costelytra zealandica*, the FD_{50} value (the concentration at which feeding is reduced to 50% of the control value) of this compound was as low as 0.03 ppm.[168] On the other hand, several flavonoids have been found to be used by monophagous or oligophagous insect species to recognize their host plants and to stimulate feeding (see Table 7.4).[240,273]

Anthocyanins embrace most of the natural red and blue pigments in flowers, fruits, and leaves.[113] They are glycosides, with glucose as the most common sugar moiety. Thus cyanin (**15**) is a glucose ester of cyanidin.

Tannins are polyphenolic compounds (molecular weight 500–20 000 daltons) that are found in all classes of vascular plant, often in high concentrations (Table 4.4). They usually occur as soluble components in the sap of living cells. Tannins bind with their phenolic hydroxyl groups to almost all soluble proteins, producing insoluble co-polymers. Enzymes complexed in this way show a marked reduction in activity. In addition, proteins bound to tannins cannot be degraded by enzymes in the digestive tract, and tannins are therefore generally thought to decrease the nutritional value of plant tissues (see Chapter 5). Tannins may also cross-link

Table 4.4 Concentrations of some secondary compounds in plants

Compound	Class	Plant species	Concentration (% dry weight)	Reference
Vincristine	Alkaloid	*Catharanthus roseus* (leaf)	0.0002	255
Sinigrin (**61**)	Glucosinolate	*Brassica oleracea* (leaf)	0.03–0.3	53
Digitoxin	Cardenolide	*Digitalis purpurea* (leaf)	0.06	188
Bergaptan (and others)	Furanocoumarin	*Pastinaca sativa* (leaf)	0.1	297
Aristolochic acid	Alkaloid	*Aristolochia philippinensis* (leaf)	0.1	185
Colchicine	Alkaloid	*Merendera montana* (leaf)	0.1	109
Glucobrassicin (**27**)	Glucosinolate	*Brassica oleracea* (leaf)	0.3–3	53
Hypericin (**31**)	Quinone	*Hypericum hirsutum* (inflorescence)	0.3	214
Hypericin (**31**)	Quinone	*H. perforatum* (leaf)	1.4	243
Aucubin	Iridoid glycoside	*Plantago lanceolata* (leaf)	0.4	180
Tomatine (**66**)	Glycoalkaloid	*Lycopersicum esculentum* (leaf)	0.5–5.1	242
Quinolizidine alkaloid	Alkaloid	*Lupinus arboreus* (leaf)	0.8–2	5
Quinolizidine alkaloid	Alkaloid	*L. arboreus* (seed)	2–14	5
Pyrrolizidine alkaloid	Alkaloid	*Senecio jacobaea* (leaf)	1.6–5.5	178
Cyanogen	Cyanogen	*Ryparosa* sp. nov. (seed)	1	287
Nicotine	Alkaloid	*Nicotiana tabacum* (whole plant)	1–6	244
Amygdalin (**4**)	Cyanogenic glycoside	*Prunus amygdalus* (seed)	3–5	98
Ligustaloside	Polyphenol	*Ligustrum vulgare* (leaf)	4.1	222
Berberine	Alkaloid	*Berberis vulgaris* (bark)	5	232
Catalposide	Iridoid glycoside	*Catalpa bignonioides* (leaf)	5.3	33
Tannins	Polyphenol	*Quercus robur* (leaf)	0.6–6	91
Tannins	Polyphenol	*Englerina woodfordioides* (leaf)	15	284
Tannins	Polyphenol	*Acer saccharum* (leaf)	30	208
Tannins	Polyphenol	*Thea sinensis* (leaf)	<30	279
L-Dopa	Amino acid	*Mucuna* (seed)	5–10	20
Pinitol	Sugar alcohol	*Mesembryanthemum crystallinum* (leaf)	10	209
Tremulacin (and others)	Phenolglycoside	*Populus trichocarpa* (leaf)	23	21, 259
Resin	Phenolic aglycones	*Mimulus aurantiacus* (leaf)	>30	122
Resin	Phenolic aglycones	*Larrea cuneifolia* (young leaves)	44	218

Table 4.5 Distribution of hydrolysable and condensed tannins in the plant kingdom (from Swain, 1979,[256] reproduced with permission)

Taxon	**% of plant genera containing tannins**	
	Hydrolysable	**Condensed**
Psilopsida (primitive ferns)	0	0
Lycopsida (club mosses)	0	0
Sphenopsida (horsetails)	0	28
Filicopsida (ferns)	0	92
Gymnosperms	0	74
Angiosperms	13	54
Monocotyledons	0	29
Dicotyledons	18	62

with nucleic acids and polysaccharides, thereby impeding their physiological function.

Tannins are commonly classified into two types: the hydrolysable and the non-hydrolysable, or condensed, tannins. Hydrolysable tannins can be hydrolysed with hot, dilute acid. They are limited to angiosperms. In contrast the condensed tannins are widespread in the plant kingdom (Table 4.5). The most common hydrolysable tannins are esters of gallic acid (**23**) and hexahydroxydiphenic acid (**30**) with sugars. Condensed tannins are polymers of flavonoid units (see Fig. 4.1), linked by carbon–carbon bonds that are not susceptible to hydrolysis. During plant tissue maturation, such as fruit ripening, tannins often polymerize further and as a result become less soluble, leading to a decrease of their astringent taste.

4.5 Glucosinolates

This is a small but well defined and basically coherent group of compounds.[90,191] Their general formula is given in Figure 4.1. All glucosinolates, or mustard oil glucosides, contain sulphur as well as nitrogen atoms. They can be either acyclic, for example sinigrin (or allyl isothiocyanate (**61**)), or aromatic, for example sinalbin (**60**). Hydrolysis of glucosinolates is facilitated by the enzyme myrosinase, which leads to the formation of isothiocyanates (or mustard oils), nitriles, and other compounds, depending on pH and other conditions. Hydrolysis occurs rapidly when plant tissue is ruptured, but also takes place, although at a much lower rate, during normal catabolism. Glucosinolates occur mainly, but not exclusively, in the Brassicaceae plant family. Since Verschaffelt's[278] historical experiments with sinigrin and cabbage white butterflies, this group of compounds has attracted much interest from students of insect–plant relationships.[191] Glucosinolates are unpalatable and toxic to many generalist feeders and to several specialists living on non-cruciferous plants.[190,215] Glucosinolates are strong feeding and oviposition stimulants to many specialists on plants belonging to the Brassicaceae family (Fig. 4.3).[50]

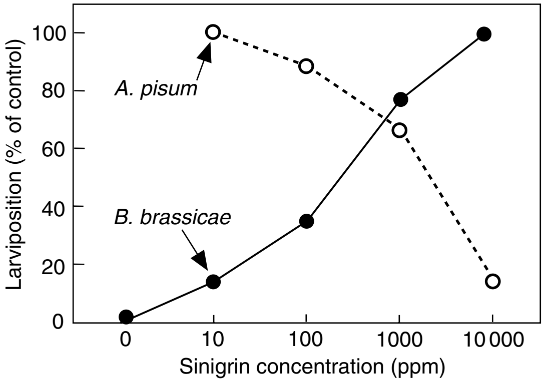


Figure 4.3 Production of larvae of pea aphids (*Acyrthosiphon pisum*) and cabbage aphids (*Brevicoryne brassicae*) on broad bean (*Vicia faba*) leaves treated systemically with sinigrin, a glucosinolate. Controls were untreated turnip leaves for *B. brassicae* and untreated broad beans for *A. pisum*. Sinigrin inhibits reproduction in species not occurring on cruciferous plants but stimulates the species specialized on crucifers, even when the compound occurs in non-host plants. (From Nault and Styer, 1972.)[199]

4.6 Cyanogenics

Probably all plants have the ability to synthesize cyanogenic glycosides (general formula in Figure 4.1), but in most species they are metabolized and not accumulated. An estimated 11% of all plants, however, contain appreciable amounts of cyanogenics. The leaves of *Eucalyptus cladocalyx*, for instance, may allocate 15% of their nitrogen content to a constitutive cyanogenic glycoside, prunasin (**48**).[40]

Cyanogenic compounds are optically active because of the chirality of the hydroxylated C-atom. Thus prunasin, originally named because of its occurrence in *Prunus* spp., is the stereoisomer of sambunigrin (**56**), typically found in *Sambucus* spp. The vacuoles of plant cells often serve as a storage place (e.g. in many Rosaceae). On damage of plant tissue, the cyanogenic compounds are enzymatically hydrolysed and very toxic hydrogen cyanide (HCN) is formed.[108] When crushing the leaves of, for example, bird cherry (*Prunus padus*) its characteristic 'bitter almond smell' is easily discernible. In addition to a probable function as storage forms for reduced nitrogen, a protective role of cyanogenics against herbivores and pathogens is appealing.[138] The fact that HCN is a potent feeding deterrent to a diverse range of insects supports its conjectural protective role.

4.7 Leaf surface chemistry

The first physical contact between an insect and a plant occurs when the insect lands or otherwise touches the leaf surface. Chemical characteristics of the plant surface, in addition to its physical features as discussed in Chapter 3, affect the insect's subsequent behaviour (see Chapter 7). Therefore, leaf surface chemistry merits special attention.

The surface waxes or resins constitute the first line of plant resistance. The structural as well as chemical composition of the epicuticular wax layer differs among plant species. There is an extensive variation in their micromorphology,[186] ranging from amorphous films to mixed arrays of wax tubes, rods, and plates. Their chemistry typically includes a variety of long-chain hydrocarbons, alkylesters, primary alcohols, and fatty acids.[88] Waxes extracted from wheat leaves, for instance, may contain up to 50 different components[46] and the waxes covering the fronds of the royal fern (*Osmunda regalis*) may consist of 139 constituents.[141] Wax composition may vary considerably, not only among congeneric species, as in the case of eight Papaveraceae species,[140] but also among different genotypes of the same species.[46]

Frequently, the wax coating contains several primary and/or secondary metabolites, albeit mostly in small amounts. When intact plants are dipped briefly into water or organic solvents[249] a wealth of compounds is often washed off. Such washings of the leaf exterior may contain sugars,[67,173] some amino acids,[67,247] and also secondary plant substances, such as phloridzin (**45**, a dihydrochalcon of apple leaves[161]), glucobrassicin (**27**, a glucosinolate from cabbage plants[272]), furanocoumarins,[250] and alkaloids (see Table 7.3).[140] These washing techniques release both intracuticular waxes, i.e. waxes embedded in the polymeric cutin matrix, and epicuticular waxes, present as a thin film on the surface of the cutin matrix. The chemical composition of both layers can be quite different.[143] A new technique allows the isolation of the epicuticular film for chemical analysis.[142] With this method it is possible to determine with increased accuracy what chemical profile insects meet when contacting the plant leaf.

As plants, like all other living organisms, represent dynamic systems it is not surprising that leaf-surface chemistry shows seasonal variation. In carrots (*Daucus carota*), for instance, the quantities of some flavonoids present on the leaf surface show large changes correlated with plant phenology. The concentrations of these secondary compounds reach a maximum during the transition from the vegetative state to the reproductive state, suggesting that factor(s) associated with changes in plant developmental state influence the transport of these compounds across the cuticle.[36]

Contact with these surface chemicals often suffices to prevent insects from further investigation of the plant. Migratory locusts, for instance, may be inhibited from taking a test bite from an intact plant merely upon palpation. When the leaf waxes have

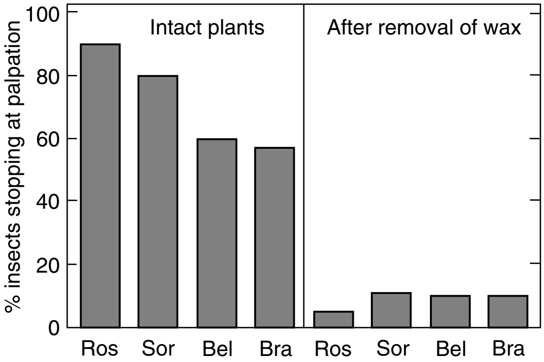


Figure 4.4 Rejection rates of four plant species at palpation by fifth instar nymphs of *Locusta migratoria*. Ros, *Rosa*; Sor, *Sorghum*; Bel, *Bellis*; Bra, *Brassica*. (From Woodhead and Chapman, 1986.)[296]

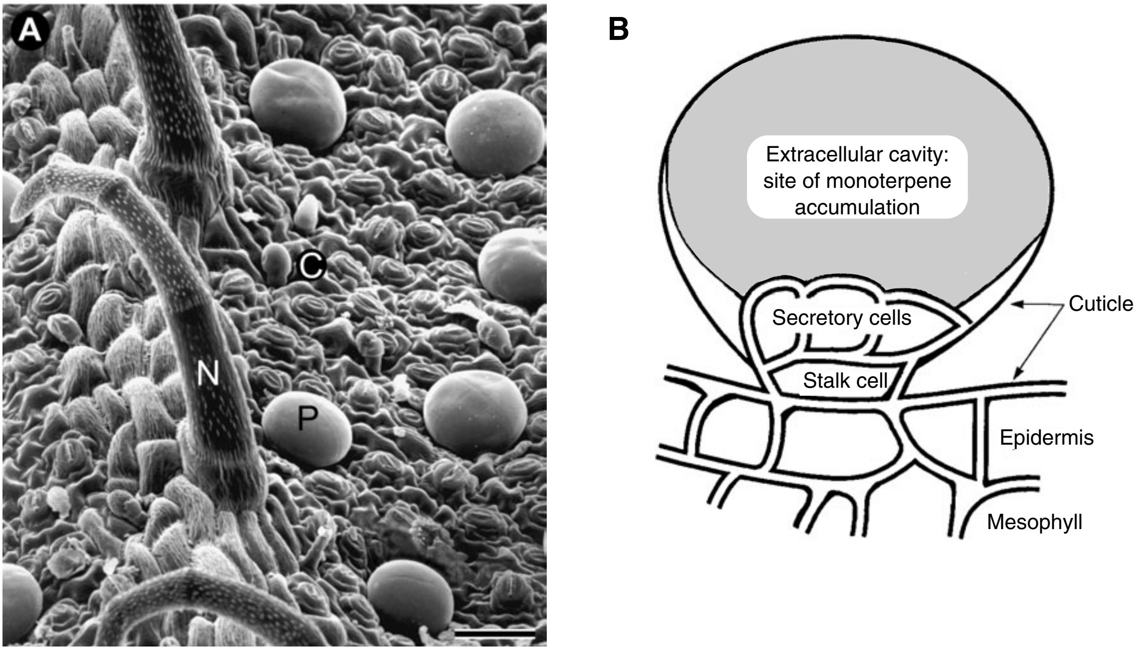


Figure 4.5 Glandular trichomes on leaf surfaces that accumulate monoterpenes. (**A**) Scanning electron micrograph of abaxial leaf surface of peppermint (*Mentha piperita*). P, peltate trichomes; C, capitate trichomes; N, non-glandular trichome. Scale = 50 μm. (Reproduced by courtesy of G. Turner, Washington State University, USA.) (**B**) Schematic cross-sectional view of a glandular trichome from the leaf surface of peppermint, showing secretory cells where monoterpenes are produced before being discharged in an extracellular cavity. (From Gershenzon and Croteau, 1991.)[105]

been removed, however, the insects take one or a few test bites before deciding to reject these non-host plants (Fig. 4.4). The other side of the coin is that leaf-surface chemicals may help some insects to recognize their specific host plants at an early stage. In several beetle species food intake is stimulated by the dominant wax components of each of their host plants.[3]

Glandular (or secretory; Fig. 4.5) trichomes are appendages of the epidermis, found mainly in the Labiatae, Solanaceae, Asteraceae, and Geraniaceae. They contain highly specialized secretory cells that synthesize and accumulate a large variety of terpene oils and other essential oils. Some glandular trichomes may permanently exude secretions soon after they are produced. Others are 'touch sensitive' and release, when ruptured, a sticky secretion in which small insects are trapped and killed (Fig. 4.6).[84,229] Larger insects are often deterred by the exudate, or their feeding is hampered, thus limiting population development.[154]

The literature on plant surface chemistry is well covered in reviews by Juniper and Southwood,[146] Chapman and Bernays,[48] Städler and Roessingh,[249] and Derridj.[66]

4.8 Plant volatiles

All plants emit a plethora of chemically divergent volatile hydrocarbons.[82] Many secondary plant substances and several intermediates of primary metabolism may be released by plants and have a sufficiently high vapour pressure to affect other organisms as a volatile. Many terpenoids, aromatic phenols, alcohols, aldehydes, etc., with molecular weights ranging from 100 to about 200 Da, easily volatilize when exposed to the air and are indeed liberated when plant tissues are damaged. Intact plants also give off such volatile compounds, which permeate through open stomata, leaf cuticles, and gland walls, but the release rate is much lower. In the past, identification of plant volatiles began with extracts of chopped or macerated plant material. During the past three decades 'headspace collection' methods have been developed to obtain the volatiles from the air around undamaged (or damaged) plants. As it reflects what is actually released from the plant into the air surrounding the plant, this technique, in combination with gas chromatography, obviously gives much more reliable information on the composition of naturally emitted blends of volatiles than the tissue extraction methods. For example, the headspace air of cotton plants contains 54 chemicals, but only six of them also occur among the 58 compounds present in the essential oil of cotton buds.[129]

Figure 4.6 When ruptured, trichomes on the leaves of a wild potato species, *Solanum berthaultii*, release a clear liquid exudate that, upon exposure to air, turns into a sticky viscous substance that acts as a natural glue for small insects. A peach aphid (*Myzus persicae*) is immobilized due to glue lumps on its feet. (Reproduced by courtesy of H.M. Smid, Wageningen University, The Netherlands.)

Plant volatiles can be classified into general and specific volatiles. The commonly occurring 'green leaf volatiles',[280] which give damaged leaves a characteristic 'cut grass' smell,[158] are mostly six-carbon saturated or monounsaturated alcohols and aldehydes, which can have different configurational isomers. Some authors also include under this heading some of their derivatives (e.g. acetates) (Fig. 4.7). They are generally produced, mostly in appreciable amounts, by oxidation of leaf lipids.[126] The precursor of the unsaturated aldehydes and alcohols, linolenic acid, often accounts for more than 1% of the dry weight of leaves. The relative amounts of the various green odour components emitted may be unique for a given plant species.

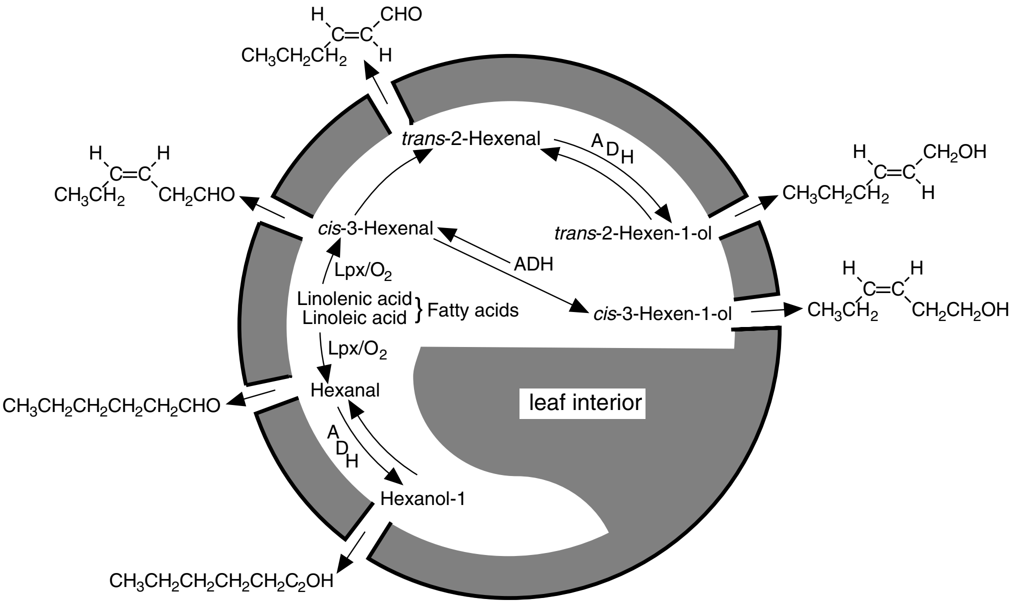


Figure 4.7 Green-leaf volatiles are derived from fatty acids in the leaf interior and permeate into the surrounding air via the stomata. ADH, alcoholdehydrogenase; Lpx, lipo-oxygenase. (From Visser and Avé, 1978.)[280]

Some insects can perceive these species-specific variations and employ them to discriminate between host and non-host plants. Colorado potato beetles, for instance, respond positively to the mixture of green leaf volatiles produced by potato foliage, but when the natural blend is distorted by raising the concentration of one of its components, the response disappears.[280]

Many, or perhaps most, plants also emanate taxon-characteristic volatiles, but so far they have been investigated with headspace techniques in only a limited number of cases. Many insects respond to a wide variety of plant-derived volatiles and use them as airborne cues in finding or avoiding certain plants (see Chapter 6).

The number of volatile substances in the air around plants may run up to several hundreds, although often the blend is dominated by one or a few major compounds (Fig. 4.8). The air around corn leaves, for instance, contains at least 24 compounds, but the major fraction (75%) consists of only seven components.[41]

In the headspace profiles that are graphically depicted in Figure 4.8, some trends can be noted with respect to the major categories of compound. In all plant species, the major headspace volatile is either a green leaf volatile (aldehyde or ester) or a terpenoid. When damaged, the proportion of this major component can either increase (soybean, eggplant) or slightly (cabbage, thale cress) or strongly (cowpea, pepper) decrease. In all plants, mite or caterpillar damage induces the release of several compounds that either are not found at all or are found in much lower proportions from intact plants. Another interesting fact is that plant species belonging to the same family may show clear differences in their emitted volatiles (compare, for example, cowpea and soybean). A number of *N*-oximes were detected only from eggplant, and two cycloheptadienes were in appreciable amounts found exclusively from thale cress. Clearly, plants change their release profiles, often dramatically, upon damage (Figs. 4.8 and 4.9).[262,282] Moreover, the bouquet emitted after mechanical damage differs from that induced by herbivore damage.[261,263]

The examples given show that large qualitative and quantitative differences often exist between the volatiles from different plant species. This may even be the case between the volatiles released by different cultivars. Thus, of 43 compounds produced by three chrysanthemum cultivars, only 14 were common to all three of them.[251] Although in this chapter the discussion is restricted to volatiles from vegetative plant parts alone, it may be mentioned that flower fragrances often consist of 100 or more components (see Chapter 12).

Damage inflicted by herbivorous insects has been found to stimulate considerably the emission of plant volatiles; the amounts emitted by herbivore-damaged plants can be nearly 2.5-fold higher than those from intact plants.[282] It is noteworthy that terpenoids often take a prominent place in the profile of blends released by damaged plants (see Fig. 4.8). Two acyclic methylene terpenes, *E*-4,8-dimethyl-1,3,7-nonatriene (**18**) and 4,8,12-trimethyl-1,3(*E*),7(*E*),11-tridecatetraene (**69**) are of special interest, as they are often found in the headspaces of herbivore-infested plants.[30] The amounts of these compounds vary with the herbivore species. Thus the headspace of apple leaves infested with the spider mite *Panonychus ulmi* contains 49% 4,8-dimethyl-1,3(*E*),7-nonatriene, whereas the proportion is only 9% when another spider mite, *Tetranychus urticae*, infests the leaves. Interestingly, these differences suffice to attract different species of predatory mite.[69] Apparently these predators react to specific ratios of odour components.

In contrast to compounds that remain inside the plant and can be recycled when necessary, chemicals that are released into the air entail a permanent loss of energy. The energy the plant has to put into the production of compounds that are either purposely or unavoidably given off is correlated with the quantity as well as the types of compound produced. The limited data available suggest that in some cases the production costs are not negligible. For example, the production of volatile isothiocyanates by *Bretschneiderea sinensis* during active growth may amount to 0.7% (expressed as a fraction of dry weight of total growth) per day.[32]

Huge amounts of hydrocarbons, in particular isoprene and monoterpenes, are emitted from woody plant species and ferns. Isoprene release in the light entails a non-trivial loss of carbon that was recently photosynthetically assimilated; immediate

Aldehydes: **1**. (*E*)-2-hexenal **2**. hexanal **3**. heptanal **4**. octanal **5**. nonanal **6**. decanal **7**. undecanal **8**. (*E*)-4-oxo-2-hexenal ***Nitrogen-containing compounds***: **9**. 2-methylbutanenitrile **10**. 5-(methylthio) pentanenitrile **11**. 6,7-dithiooctanenitrile **12**. 2-methylpropanal, *O*-methyloxime **13**. (syn) or (anti)-2-methylbutanal, oxime **14**. 2-methylbutanal, *O*-methyloxime **15**. 3-methylbutanal, *O*-methyloxime **16**. phenylacetaldehyde, *O*-methyloxime (tentative) ***Ketones***: **17**. 2-butanone **18**. 4-methyl-3-penten-2-one **19**. 4-hydroxy-4-methyl-2-pentanone **20**. 1-penten-3-one **21**. 3-pentanone **22**. 4-methyl-3-heptanone ***Alcohols***: **23**. (*Z*)-3-hexen-1-ol **24**. 1-hexanol **25**. 2,4-pentanediol, 2-methyl **26**. 1-octen-3-ol **27**.1-penten-3-ol **28**. 2-penten-1-ol **29**. 1-pentanol **30**. 2-ethyl-1-hexanol **31**. 1-nonanol **32**. 1-dodecanol ***Carboxylic acids***: **33**. hexanoic acid ***Esters***: **34**. (*Z*)-3-hexen-1-ol, acetate **35**. hexyl acetate **36**. methyl salicylate **37**. (*Z*)-3-hexen-1-ol, butanoate **38**. hexyl butanoate **39**. (*Z*)-3-hexen-1-ol, 2-methylbutanoate **40**. (*Z*)-3-hexenyl acetate ***Sulphides***: **41**. dimethyldisulphide **42**. dimethyltrisulphide ***Terpenoids***: **43**. linalool **44**. limonene **45**. (*E*)-β-ocimene **46**. α-pinene **47**. (3*E*)-4,8-dimethyl-1,3,7-nonatriene **48**. (3*Z*)-4,8-dimethyl-1,3,7-nonatriene **49**. α-bergamotene **50**. (*E*)-β-elemene **51**. (*Z*)-β-elemene **52**. (*E*,*E*)-α-farnesene **53**. (*E*)-β-farnesene **54**. germacrene A **55**. α-selinene **56**. β-selinene **57**. (3*E*,7*Z*) or (3*Z*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene **58**. (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene **59**. α-thujene **60**. β-pinene **61**. sabinene **62**. myrcene **63**. β-phellandrene **64**. 1,8-cineole **65**. (*E*)-sabinene hydrate **66**. longifolene **67**. β-ionone ***Others***: **68**. 6-[(*Z*)-1-butenyl]-1,4-cycloheptadiene **69**. 6-butyl-1,4-cycloheptadiene **70**. tetradecanol

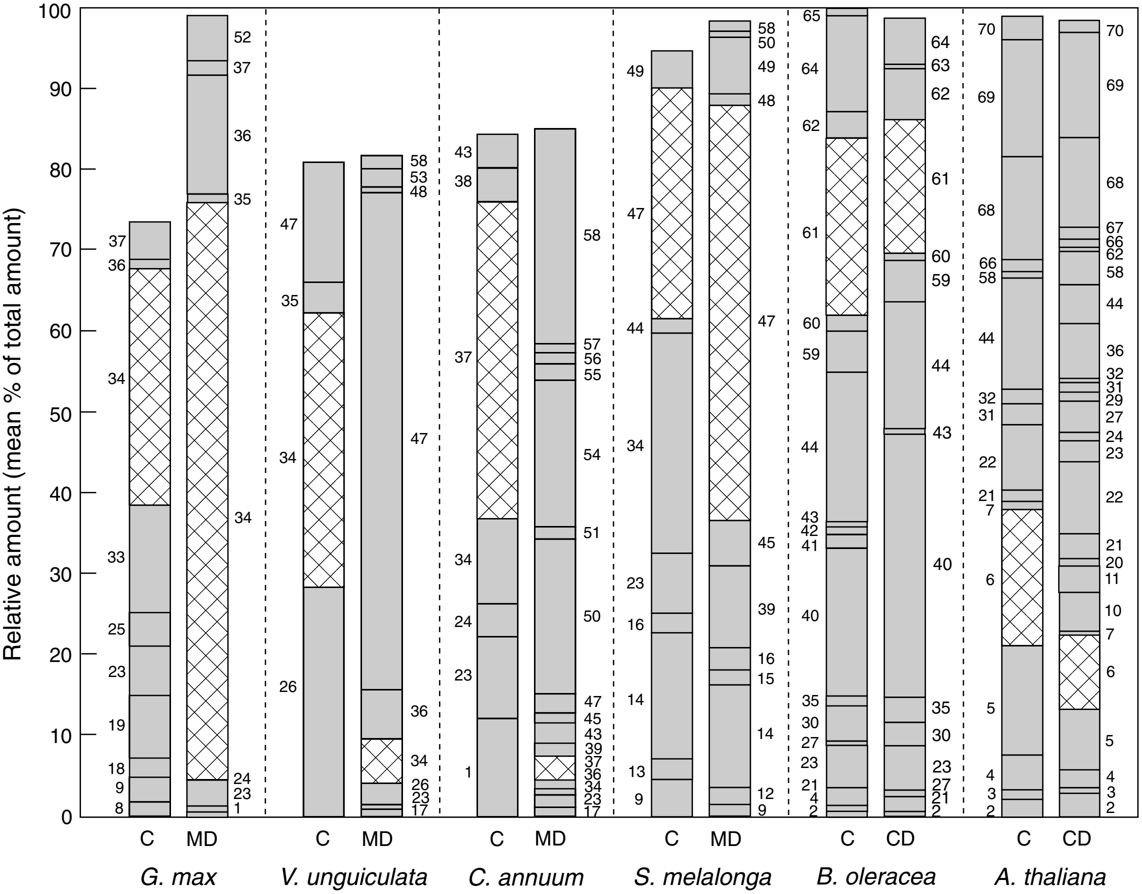


Figure 4.8 Headspace analysis of six plant species belonging to three families. Headspace composition of clean leaves (control, C) are compared to leaves damaged by spider mites (*Tetranychus urticae*) (MD) or caterpillars (*Pieris rapae*) (CD). All compounds that represent at least 0.5% of the total amount of the volatile blend are depicted for soybean (*Glycine max*), cowpea (*Vigna unguiculata*) (Fabaceae), sweet pepper (*Capsicum anuum*), eggplant (*Solanum melalonga*) (Solanaceae), Brussels sprouts (*Brassica oleracea*), and Thale cress (*Arabidopsis thaliana*) (Brassicaceae). The major component in the volatile blend of the undamaged control of each plant species is cross-patterned to accentuate the differences between intact and herbivore-damaged plants. (Data from Van den Boom *et al.*, 2004;[271] Blaakmeer *et al.*, 1994;[26] Van Poecke *et al.*, 2001.)[276]

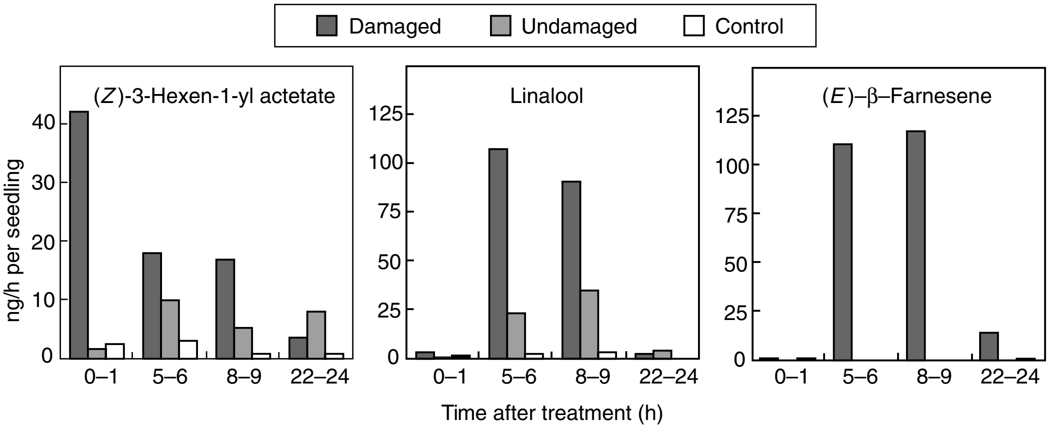


Figure 4.9 Amounts of three components of leaf volatiles emitted by corn seedlings after artificial damage followed by treatment with caterpillar regurgitant (to mimic herbivory) at various times after treatment ('damaged'). Some components are also, albeit with some delay, released systemically by 'undamaged' leaves of injured plants. Volatiles released by unharmed plants were used as a 'control'. Note that the composition of odour blends emitted upon damage changes with time. (Data from Turlings and Tumlinson, 1992.)[262]

losses are estimated to range typically between 0.5% and 2%, but are much higher at high temperatures.[123]

4.9 Concentrations of secondary plant substances

The relative amounts of secondary compounds found in plants not only vary spectacularly (see Table 4.4) but are also quite heterogeneously distributed over the various plant parts. The latter point is very relevant to herbivorous insects, because they often feed on particular cells or certain tissues only (see Chapter 2). From the plant's point of view it seems logical, when a protective function is attributed to secondary plant substances,[95,96] to allocate most of its defensive chemicals to those parts where insect damage would inflict the greatest losses in plant fitness.[270] Different plant parts would store different levels of protectants, because damage to seeds, for instance, would have a greater impact on plant fitness than damage to old leaves. Fruits of wild parsnip (*Pastinaca sativa*), in accordance with this concept, harbour four times higher furanocoumarin concentrations than leaves and 800 times higher levels than roots.[297] Likewise, the flowers of *Hypericum hirsutum* contain five to ten times more hypericin than the leaves,[214] and young leaves and other growing plant parts are generally better protected by secondary compounds than mature tissues. Thus the youngest leaves of rosette plants of hound's tongue (*Cynoglossum officinale*) contain up to 53 times higher levels of pyrrolizidine alkaloids than older leaves (Fig. 4.10).[268]

Even the leaves of the same tree branch may differ in, for instance, polyphenol contents to such an extent that tree-dwelling caterpillars make extensive foraging trips within the canopy, feeding on some leaves only and avoiding others. Clearly many forest insects, like lepidopteran and sawfly larvae, make careful choices as they forage.[100,234]

Even within a single leaf, local concentrations of protective chemicals may vary and, in poplar leaves for instance, gradually increase from the base to the leaf tip (Fig. 4.11). Colonizing gall aphids, therefore, do not settle randomly on a leaf, but nearly always attempt to form their galls at the base of that leaf, where the concentration of phenolics is lowest.[298] Likewise, nicotine gradients in leaves of tobacco plants showed a 2–3-fold increase from the basal to apical portion of the leaf, and usually from medial to edge. Tobacco hornworms (*Manduca sexta*) prefer to feed on the low-nicotine leaf regions, whereas tomato hornworms (*M. quinquemaculata*) show the opposite response.[156]

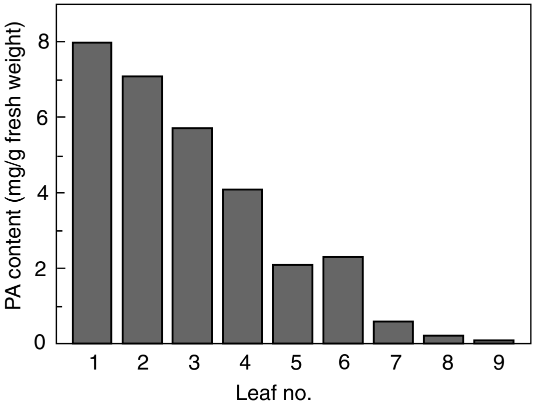


Figure 4.10 Concentration of pyrrolizidine alkaloids (PAs) per leaf of *Cynoglossum officinale* rosette plants. No. 1 is the youngest leaf. (From Van Dam *et al.*, 1994.)[268]

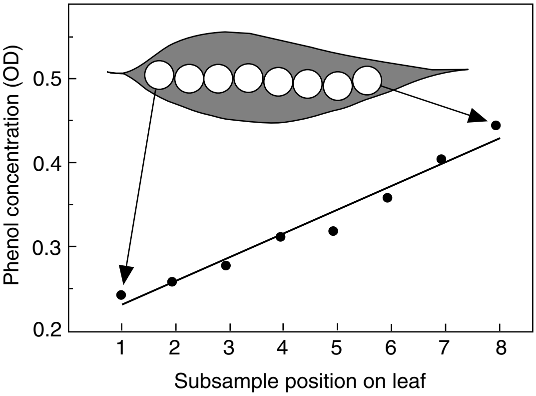


Figure 4.11 Concentration of phenolics (expressed as optical density, OD) in eight subsamples of a single leaf blade of *Populus angustifolia*. Gall aphids (*Pemphigus betae*) prefer to form their galls at the base of the leaf, where the concentration of phenolics is lowest. (Redrawn from Whitham, 1983;[289] data from Zucker, 1982.[298])

Another example in which different elements of a plant organ show strikingly different quantities of secondary compounds is provided by *Cola nitida*. The caffeine (**9**) content of its pod husk is only a trace, that of the seed coat is 0.44% (dry weight), but that of the seed contents is 1.85%.[204] Not only different leaf parts, but also different leaf tissues, often show considerable quantitative differences in resistance-conferring chemicals. As neonate larvae, when they start to feed, often first encounter the contents of epidermal cells, it seems a good strategy for a plant to concentrate its chemical resistance in its epidermis. In Poaceae, cyanogenic glucosides are concentrated in epidermal cells to such an extent that they represent 90% of the soluble carbohydrate content of the epidermal tissue.[211] The palisade layer in the mesophyll of American holly (*Ilex opaca*) leaves consists of 38% (dry weight) of saponins, whereas the remainder of the leaf contains on average only 1.3%. Despite these huge amounts of saponins in the palisade cells, which act as protease inhibitors in the guts of many herbivores, larvae of *Phytomyza ilicicola* tunnel exclusively in this particular tissue, which has a protein concentration of about 10 times that of the remainder leaf tissues.[157] In contrast, some leaf-mining larvae feeding on oak trees prefer the spongy mesophyll cells and avoid the palisade layer, which is in this case high in tannins.[91]

In a number of cases secondary plant substances are produced in tissues other than where they accumulate. Some alkaloids and other compounds, for example nicotine (**41**) in tobacco plants, are synthesized in the roots and transported via the xylem to the leaves.[15] Alkaloids often occur at the highest concentrations in young growing plant tissues.

Of course, the quantities of secondary compounds vary between individual plants. These differences are often considerable, not only quantitatively but to some extent also qualitatively. There is a 20-fold inter-individual variation in cyanogenic glycoside content in the foliage within a Costa Rican population of *Acacia farnesiana* occupying a few hectares, whereas the flavonoid content of these same leaves stays constant in kind and quantity.[238] Such variations are probably of great ecological significance, although only relatively recently have biologists realized that herbivores may behave quite differently on different plant individuals belonging to the same species.[150]

Given that a single plant can contain hundreds of constituents in its essential oils alone, the biosynthetic versatility of plants, which probably goes back to very ancient life forms,[45,223] is difficult to encompass. Clearly, the enormous diversity of secondary plant compounds is based upon the fact that plants generally contain mixtures of many compounds rather than just one or a few characteristic substances. Young tea shoots, for instance, contain more than 24 phenolic compounds,[230] and the terpenoid mixtures in leaves of essential oil plants and in resin-producing trees are usually composed of 30 to 40 terpenoids each of at least 1% concentration.[169]

When biochemical versatility in the plant kingdom is studied at a geographical level, some patterns emerge. Several studies have shown a strong latitudinal gradient in the quantity and diversity of secondary compounds. Whereas about 16% of temperate plant species contain alkaloids, this value increases to more than 35% for tropical species. The amounts of condensed tannins in mature leaves are almost three times higher in tropical forests, although simple phenolics do not seem to vary between latitudes. Striking developmental differences show up when comparing concentrations of secondary compounds in young and mature leaves. In tropical trees, young expanding leaves contain much higher concentrations of phenolics, terpenes, and alkaloids than mature leaves, whereas young leaves of temperate trees, for instance, contain half the concentration of condensed tannins compared with mature leaves.[86]

The biochemical richness briefly depicted above emphatically indicates that plants, in addition to their structural and temporal characteristics, possess an extra dimension—that of chemical composition.

4.10 Production costs

Production of secondary plant substances requires matter and energy. Especially when plants contain appreciable quantities of secondary compounds, their synthesis and storage presumably exact a cost, but this has been hard to measure. The physiologist can express the costs of chemical resistance in biochemical terms, for instance the energy required to produce a certain quantity of secondary metabolites. Results for various groups of compounds, based on this method, are shown in Table 4.6, which shows that the production costs of secondary compounds are somewhat higher than those for most primary metabolites.

Terpenoids are especially expensive to produce because of their high level of chemical reduction and the often high number of enzymatic conversions involved in their formation. Several monoterpenes need nine steps, whereas the formation of the iridoid glycoside antirrhinoside, for instance, requires as many as 23 steps.[103] Of course, the total costs of chemical resistance depend not only on costs of synthesis (which are relatively small), but also on the actual quantities of the chemicals present in the plant, their turnover rates, costs of transport and storage, and costs of avoiding autointoxication. The costs of the 'handling' processes are probably appreciable. The values presented in Table 4.6 compare remarkably well with those measured in the plant *Diplacus aurantiacus* under natural conditions. A negative correlation was found between the amount of phenolic resins that coat the leaves of this species and growth rate. For each gram of resin produced there was a reduction in growth of 2.1 g dry weight shoot biomass.[117] Thus, resin production is more costly than growth.

Ecologists may determine the loss of fitness due to the commitment of resources to defensive chemicals and focus on the adaptive value of secondary compounds in terms of plant organ or tissue value, reduction in growth or seed production, apparency to herbivores, etc. This method was applied in a study of the growth of young trees of *Cecropia peltata*, which commonly occurs in the

Table 4.6 Costs of the formation of various primary and secondary plant compounds, expressed as grams of glucose per gram of compound

	Cost
Primary compounds	
Carbohydrates	1.07
Organic acids	0.73
Lipids	3.10
Nucleotides	1.59
Amino acids	2.09
Secondary compounds	
Terpenoids	3.18
Phenolics	2.11
Alkaloids	3.24
Other nitrogenous secondary compounds	2.27

Average values are presented per group of compounds. For more detailed information, see Gershenzon (1994b),[104] on which this table is based.

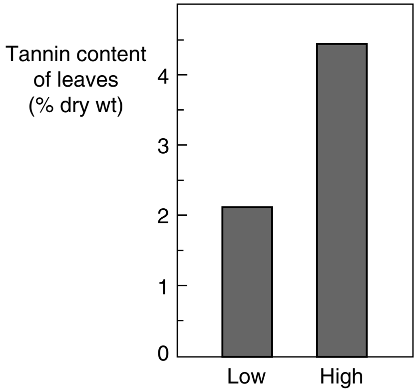
neotropics. An inverse relationship was found between growth rate and the concentration of secondary substances (tannins) present in the leaves (Fig. 4.12).

The antiherbivore effect of tannins was demonstrated in the same study. When 18-month-old plants grown under standard conditions but varying in tannin content were placed in a large forest light gap and for 10 days subjected to naturally occurring herbivores, damage to low-tannin plants was significantly higher than damage to high-tannin plants. The investment in tannin production often appears to be quite substantial. In *Cecropia* the increase in tannin content from 1% to 6% translates into a greater than 30% reduction in the rate of leaf production.[55] Likewise, birch trees (*Betula pendula*) show a negative correlation between tree height and amounts of flavonols in the leaves, indicating that anti-herbivore substances are produced at the expense of growth.[197]

It should be remembered that secondary plant substances seldom have, or more probably never have, only one function. Reduced insect infestation, as measured in *Cecropia peltata* with increased tannin levels, is probably only one beneficial effect. It seems plausible that there is a balance between investment and profit, which may consist of several components.

Results from a number of studies indicate that secondary plant metabolites are costly to manufacture,[104,281] although some other studies have presented evidence that the costs of chemical resistance are small or even absent.[253,269] Such contradictory conclusions may be due to the use of different criteria to measure plant fitness. For instance, resistance in barley (*Hordeum vulgare*) to green-bugs appeared to be costly when plant biomass growth was used as a parameter, but when the number of leaf primordia differentiated on the apex (an indicator of future growth) was used, the reverse conclusion could be drawn.[44] Clearly more experimental data and theory development are needed for a full grasp of the costs of resistance to herbivory.[241]

It should be realized that plant growth is often limited by nitrogen availability rather than energy (photosynthesis, glucose). In that case the production of nitrogen-containing compounds, such as alkaloids, comes at the expense of growth or reproduction (Fig. 4.13). This may explain why alkaloid levels are usually lower than those of, for instance, phenolics. The degree of 'protection' provided by a given chemical is another ecological



	Low	High
Leaf production (no. of leaves/18 months)	48	40
Leaves lost to insect feeding (% leaf surface eaten/day)	0.62	0.49

Figure 4.12 Comparison of leaf production and susceptibility to insect feeding on young *Cecropia peltata* trees with low and high tannin content. See text for further details. (Data from Coley, 1986.)[55]

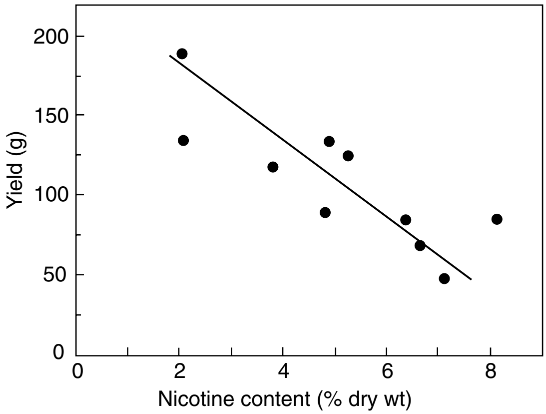


Figure 4.13 Relationship ($r = -0.86$) between the yield of foliage and nicotine content in eight native or primitive and two domesticated cultivars of *Nicotiana tabacum*. (From Vandenberg and Matzinger, 1970.)[277]

factor that determines the amounts produced. In other words, when alkaloids are generally more toxic than phenolics one would expect lower alkaloid concentrations compared with levels of phenolics.

An analysis of 33 recent studies on costs of plant resistance to herbivory showed that in the majority of cases appreciable costs are involved.[253] It should be realized, however, that investment in producing chemical resistance factors may not be an investment totally separate from other investments by the plant. Some of these investments in other plant functions may amplify the efficacy of the chemical defence system, whereas others may attenuate it.[128]

The topic of production costs has been discussed in lucid reviews by Gershenzon[104] and Simms.[241]

4.11 Compartmentation

One problem for a plant adopting a chemical resistance strategy against herbivores or pathogenic intruders is that any chemical toxic enough to be effective against a variety of organisms is likely to be self-toxic as well. This problem can be solved in two ways:

1. Instead of accumulating highly toxic compounds, the plant stores less toxic precursors, which are transformed into toxins only when needed, for instance when damaged by herbivores.
2. Toxic chemicals are stored in cell compartments that are remote from metabolism (i.e. cell walls and vacuoles).

Both mechanisms do indeed commonly occur, often in combination with each other. Concentrations of toxic compounds in vacuoles are often extremely high. Berberine alkaloids (**7**), for example, occur at levels of more than 0.25 M in cell vacuoles of the greater celandine (*Chelidonium majus*). Employing specialized storage sites requires physiological machinery to transfer the compounds from their place of synthesis, and the presence of specific membrane-carriers to accumulate them in the storage organs and to prevent them from 'leaking' away from these sites. Several mechanisms have been described that execute such uphill transports.[294] Obviously these processes entail metabolic costs.

By binding to sugars, the toxicity of many compounds is diminished and their solubility is increased, so that large amounts can be stored in the cell vacuoles. It is only upon leaf damage that such glycosides come together with specific degradation enzymes to produce the poison. Young shoots of sorghum (*Sorghum bicolor*) may contain up to 30% (dry weight) dhurrin (**16**), a cyanogenic glycoside. Most of it is stored, at still higher concentrations, in the vacuoles of epidermal cells. The chloroplasts of the mesophyll cells contain the enzyme β-glucosidase, and the cytosol of these cells holds hydroxynitrile lyase. When the leaves are damaged dhurrin is mixed with these two enzymes, resulting in immediate degradation and production of hydrogen cyanide (Fig. 4.14).[108] This compound is well known for its general toxic effect on most living organisms. Likewise, coumarin is formed by hydrolysis when white melilot (*Melilotus alba*) leaves are injured (Fig. 4.14). In *Melilotus*, self-toxicity is prevented by two membranes, which serve as barriers between substrate and enzyme: the tonoplast (vacuolar membrane) and the plasmalemma. Glucosinolates are located in the vacuole of the cell and occur throughout all organs of the plant, whereas myrosinases are localized in scattered myrosin cells, which seem to be glucosinolate free. Intact glucosinolates are non-toxic but, upon tissue damage, such as that caused by chewing insects, they come into contact with myrosinases from neighbouring cells. The glucosinolates are then converted to mustard oils, which are repellent and potentially toxic to many insect species (Fig. 4.14).[183,291]

Another form of compartmentation is exemplified by the accumulation of low-molecular-weight terpenoids and other volatile oils in glandular hairs and idioblasts, resins in resin ducts, and latex in cells called laticifers.[85] More than 20 000 angiosperm species, belonging to 40 families, are known to produce latex, an aqueous suspension or emulsion that may contain different rubbers, resins, essential oils, etc.[171] Like resin ducts these specialized containment structures are wholly devoted to the storage and excretion of toxins.

Although many secondary plant substances are stored in vacuoles, they are not inert end products and wholly disconnected from the plant's metabolic

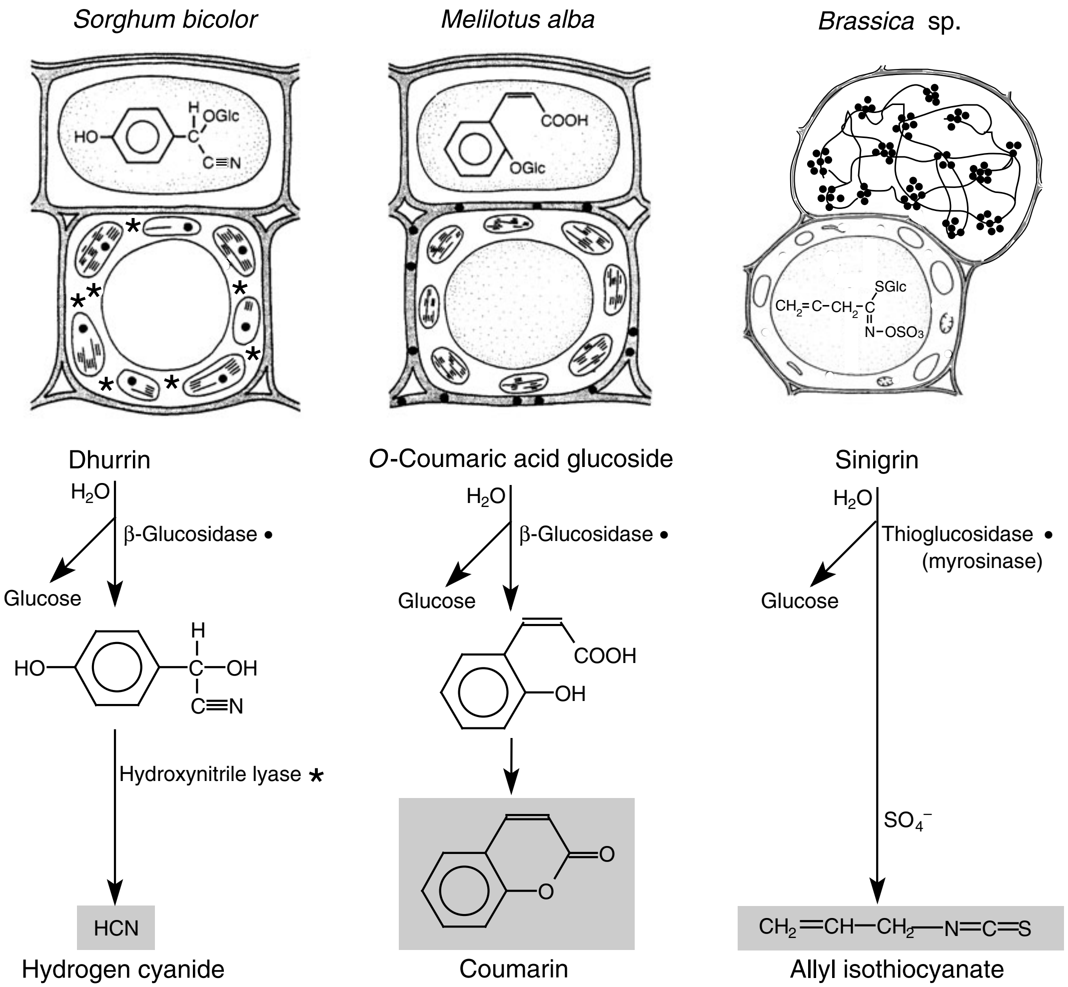


Figure 4.14 Compartmentation of precursors of toxic plant compounds and their degrading enzymes in three plant species: sorghum, white melilot, and horseradish. The glucosidases (•) are present in all three plants, but localized in different cell compartments of mesophyll tissue. Another enzyme, hydroxynitrile lyase (*), occurs in mesophyll cells of sorghum. (Redrawn from Matile, 1984.)[183]

processes. Rather, the cell vacuole forms part of a dynamic environment from which metabolites can re-enter the cytoplasm, so that this mechanism is not necessarily one of chemical disposal.

Large diurnal variations in the levels of secondary compounds support the view of a continuous metabolic involvement regulated by endogenic (e.g. developmental stage) and environmental (e.g. season, climate, amount of light) factors. Recent evidence, however, suggests that turnover rates of alkaloids and terpenes are in many cases much smaller than thought earlier.[103,125,233] The finding that phenolic glycosides and tannins/phenolics in aspen seedlings undergo significant turnover,[160] whereas salicylates in willow plantlets do not,[227] indicates that generalizations about this aspect of secondary plant compounds are still premature.

4.12 Temporal variability

The chemical make-up of a plant is not a constant and fixed property but may show extensive temporal variability (Fig. 4.15).[165] Mature plants differ in many respects from young plants, and senescing

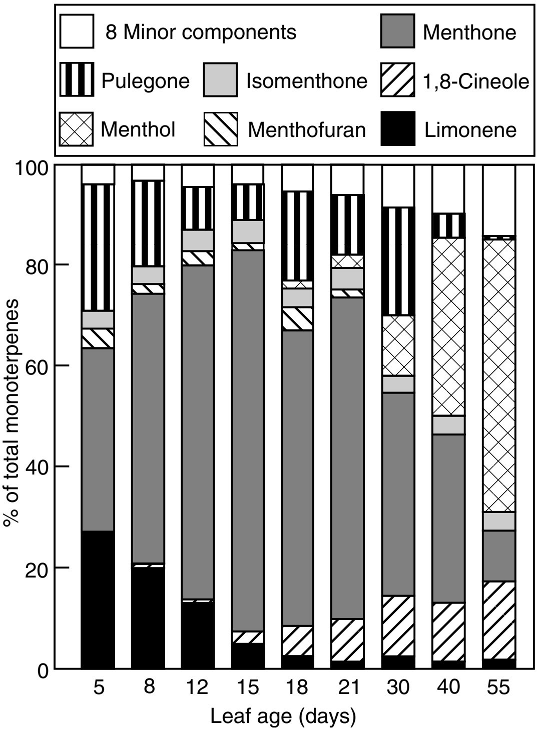


Figure 4.15 Changes in monoterpene composition during leaf development in peppermint. (Data from Gershenzon *et al.*, 2000.)[106]

plant tissues again show qualitative and quantitative changes compared with full-grown life stages. In many parts of the world these changes parallel the seasons. This is quite obvious in the ageing processes of annual species, but perennials that synchronize their reproductive periods and show morphological and physiological adaptations, such as leaf abscission and nutrient storage, also continuously change their chemical profiles. Physical factors, such as leaf toughness,[170] surface roughness,[186] and water content,[91] exhibit considerable changes with age, but the changes in primary metabolites as well as secondary substances are often even more dramatic.

4.12.1 Seasonal effects

Variations in leaf nutrients and allelochemical content with season are of paramount importance to insects feeding on them. Feeny, in a classical study, found that most insect species living on oak leaves concentrate their feeding in early spring because the nutritional value of leaves at that time is highest and declines as they mature.[91] Thus, water and protein contents decrease and tannins accumulate during the summer (Fig. 4.16). A recent study has corroborated Feeny's conclusion by showing that the abundance and richness of both early and late season fauna correlate negatively with foliage concentrations of condensed tannins.[94]

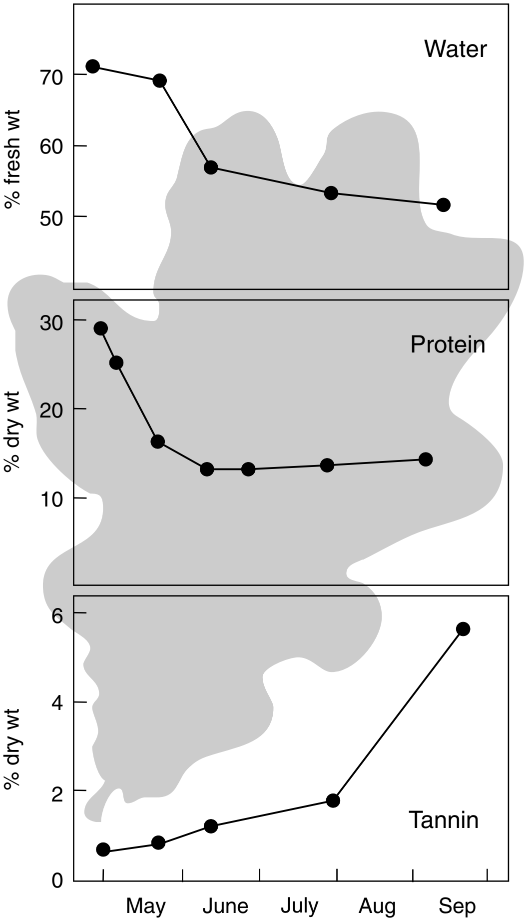


Figure 4.16 Seasonal variation in the water, protein, and tannin content of sun leaves of oak, *Quercus robur.* (Redrawn from Feeny, 1970.)[91]

The rapid increase of phenolics, reaching spectacularly high levels in spring leaves of poplar (*Populus trichocarpa*) (Fig. 4.17), undoubtedly has a physiological impact on its insect attackers. In

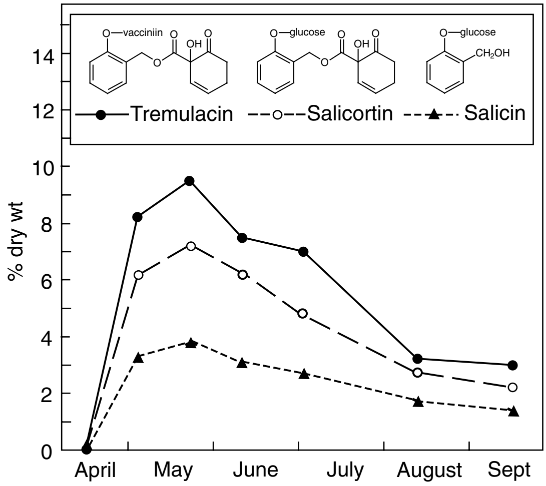


Figure 4.17 Seasonal variation in the concentration of three phenolics in the leaves of *Populus trichocarpa*. (Redrawn from Thieme and Benecke, 1971.)[259]

contrast, during winter St John's wort (*Hypericum perforatum*) plants contain 20 times more alkaloid than during summertime.[248]

Likewise, terpenoid levels in many plant species are much higher in young leaves than in mature, fully expanded leaves. Similar patterns of change occur in other organs, such as stems and roots, with young organs often having terpenoid concentrations 2 to 10 times as high as those of mature organs.[105] There is usually further surge in the production of secondary plant substances associated with the onset of flowering and seed production. Many insects, however, grow better and attain higher fecundity levels when feeding on young leaves compared with mature or senescent leaves of the same plant, because of their higher nutritional value,[139] although the opposite reactions can also be found. Thus, larvae of *Pieris rapae* prefer to feed and grow better on young cabbage leaves than on mature leaves, whereas the cabbage looper (*Trichoplusia ni*) prefers mature leaves.[35] Piercing–sucking insects may encounter still larger variations in the nutrient content of their hosts than mandibulate species.[231] A striking example is provided by willow trees. Their phloem sap contains 0.4% free amino acids during springtime, a value that decreases to about 0.05% during the summer.

Whereas many allelochemicals classified as toxins or deterrents decline in concentration with leaf age, compounds designated as digestibility-reducing or quantitative resistance factors generally exhibit the opposite pattern. These substances, such as tannins and resins, increase in many instances with leaf age and may render leaves of all plant growth forms less suitable for herbivores. The resistance-conferring compounds of early-season foliage are often nitrogen-based substances such as alkaloids, cyanogenic compounds, and non-protein amino acids. This may be related to the increased soil levels of nitrogen early in the season, and plants may use the nitrogenous compounds not only for protection, but also to store nitrogen for later growth. At the same time, carbon is limiting in the young growing tissues but later in the year carbon supply may exceed the demand for growth, permitting the plant to produce carbon-based quantitative resistance compounds such as tannins. The 'resource availability hypothesis'[56] (see Chapter 11) has been proposed as an attractive explanation for many recorded seasonal changes in nitrogen- and carbon-based resistance compounds, but its validity has recently been disputed.[201]

4.12.2 Day/night effects

On another timescale, levels of many secondary plant substances appear to fluctuate daily. Because of changes in photosynthetic and metabolic activity of their food plants, herbivorous insects are confronted with a diet during the night that differs markedly from that available during the daytime. The amounts of secondary compounds may fluctuate by as much as 35% during a day–night cycle, as has been reported for cyanogenic compounds in cassava.[205] Still larger diurnal variations were seen for quinolizidine alkaloids in white lupin (*Lupinus albus*) (Fig. 4.18). Diurnal variation has also been reported for the amount of volatiles released.[174]

Clearly, the chemical composition of plants is not constant throughout the day, but varies markedly, not only in absolute amounts of particular secondary plant substances but also in the ratios of different compounds.[220] Such diurnal fluctuations may provide a rationale for some of the ancient rules of drug plant harvesting. Theophrastos

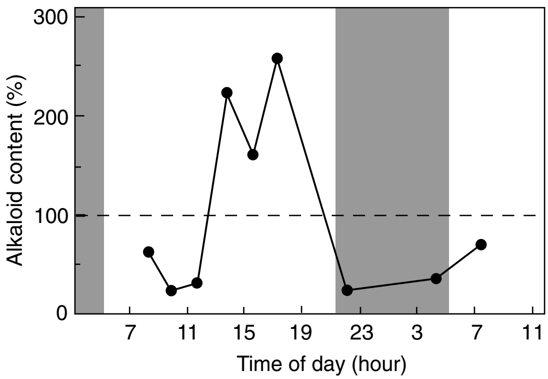


Figure 4.18 Diurnal fluctuation of lupanine and related quinolizidine alkaloids in the leaves of *Lupinus albus* as a percentage of daily average content. Dotted line = 100% level = daily average = 0.07% fresh weight. (Modified from Wink, 1998.)[293]

reports that the herb gatherers of his time (fourth century BC) prescribed that 'some roots should be gathered at night, others by day and some before the sun strikes them.'[220] Developing capsules of *Papaver somniferum*, for instance, may at 9 am yield four times more morphine in their latex than at 9 pm. Of course primary metabolites also exhibit strong daily fluctuations. Diurnal changes in the sugar concentrations of leaves have been documented in many plant species, and free amino acid levels in phloem sap may vary by a factor of 3.[101]

Diurnal fluctuations of the kind documented above may also be one of the reasons why many insects are nocturnal feeders and other species choose to feed on different plant parts at different times of the day.[153] Some instances are known of caterpillars that shift in their late instars from a day-feeding to a night-feeding habit.[59] It is not known, however, whether food quality or other ecological factors determine such foraging strategies. Food quality does play a role in the polyphagous larvae of the gypsy moth (*Lymantria dispar*), which normally forage at night. They abandon their diurnal rhythm on nutritionally poor host plants and then eat intermittently throughout the day and night. When this insect is grown on an artificial diet, feeding is also restricted largely to the night, except when the diet contains 2% tannin, in which case feeding also occurs during the daytime. Loss of the feeding rhythm under such conditions is probably an adaptation to defoliation-induced changes in food quality such as would occur under population outbreak conditions.[167]

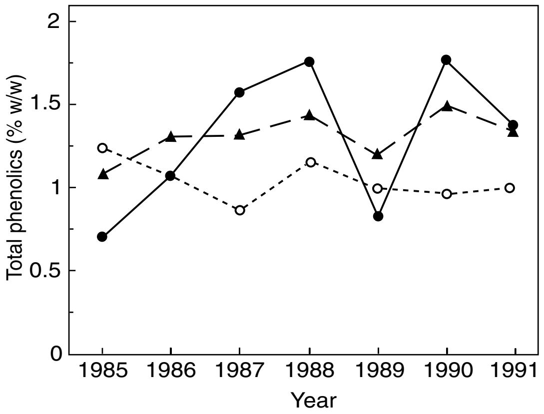


Figure 4.19 Average total phenolic content plotted by year for three grass species: *Andropogon scoparius* (●), *A. hallii* (▲), and *Carex heliophila* (○). (Redrawn from Mole and Joern, 1993.)[192]

4.12.3 Interyear variation

The role of external factors, such as climate and availability of nutrients, on chemical variation is manifested in interyear variations in the quality and quantity of allelochemicals produced by a perennial plant. This is exemplified by appreciable variations among years in the amounts of phenolics produced in three gramineaceous species (Fig. 4.19). In one grass species, *Andropogon scoparius*, the highest concentration observed was as much as 2.5 times that of the lowest.[192]

4.13 Effects of location and fertilizers

Site factors that may greatly influence a plant's chemistry include exposure to direct sunlight and physical and chemical soil characteristics.

4.13.1 Sun and shade

Light is a basic requirement of all green plants, which exist by virtue of their capacity to convert solar energy into organic matter. No wonder that light intensity generally affects plant primary and/or secondary metabolism. A reduction in light intensity will negatively affect photosynthesis with a consequent decline in carbohydrate production.

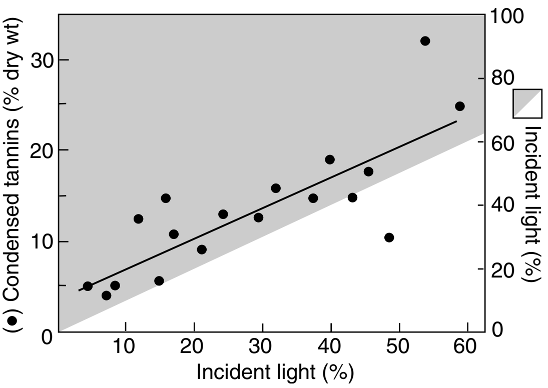


Figure 4.20 Foliar content of condensed tannins versus the percentage of available light reaching the leaves of *Acacia pennata* trees. (Data from Mole *et al*., 1988.)[193]

When mineral nutrient uptake is not affected, the result will be a net decrease in the C/N balance in shaded plants or plant parts. This will lead to lower levels of C-based metabolites, such as phenolics (Fig. 4.20).[83,193] Often shading also affects morphological traits, such as leaf toughness and the spacing of leaf veins. Shaded leaves are typically larger and thinner than sun leaves.

Many instances are known of marked differences between plants growing in the open and in the shade. For example, bracken fern (*Pteridium aquilinum*) growing in shady areas may contain up to 50% more cyanogenic compounds than those in open sites, whereas higher concentrations of flavonoids and tannins are found in plants growing in sunny spots. These differences have noticeable effects on plant palatability to insects as well as mammalian herbivores.[58] Even different parts of the same plant sometimes show significant differences in their chemical composition owing to differences in exposure to the sun.[254] As mentioned earlier (Section 2.1), in a choice test larvae of the gypsy moth preferred alder leaves picked from the south side of a tree over those taken from the north side of the same tree (see Fig. 2.5). Likewise, another notoriously polyphagous insect, the Japanese beetle (*Popillia japonica*), prefers to feed on sun-grown foliage over shade-grown foliage, thus confirming the view that generalists do show food discrimination.[226] Larvae of the polyphagous forest tent caterpillar *Malacosoma disstria* also prefer to feed on foliage of sugar maple (*Acer saccharum*) that has been exposed to sun versus exposed to shade, due to, among others, the presence of higher levels of soluble sugars.[208] The opposite response was found for larvae of the polyphagous geometrid *Epirrita autumnata* on shaded branches of its host tree, mountain birch. In this case insects grew better on shaded leaves that had higher levels of protein-bound and free amino acids and higher water content than non-shaded leaves. At the same time shaded leaves showed lower levels of total phenolics and lower toughness.[133] These examples suffice to show that shading does modify leaf chemistry, and consequently may affect herbivore performance.

Piercing–sucking insects also respond to host-plant variations resulting from differences in sun exposure. Eucalyptus trees are conspicuously more damaged on the sunny than on the shady side by the psyllid *Cardiaspina densitexta*, because the nymphs on the shaded side are exposed to a nutritionally suboptimal sap composition. This results in severe mortality.[288]

A case in which shaded foliage experiences relatively more herbivory by insects is found in bittercress (*Cardamine cordifolia*). Chemical analysis of the foliage of plants growing in the sun and of individuals from the same clone that were shaded experimentally showed considerably higher sugar levels but lower protein concentrations in the sun. Damage caused by larvae of a leaf-mining insect (*Scaptomyza nigrita*) was twice as high in shaded leaves as in sun-exposed leaves. As the levels of glucosinolates, the secondary compounds characteristic of this plant species, did not differ between shaded and sun leaves, it is concluded that the insect reacted positively to the increased protein levels in the shade.[57]

High light intensities usually stimulate secondary metabolism. As a result, whole plants, or plant parts, contain greater amounts of secondary metabolites in sunny areas than in the shade. A striking example is found in the concentration of diterpenoids, and also of total diterpene resin content, in Scots pine (*Pinus sylvestris*), which may increase by 100% in insolated needles compared with shaded foliage.[115] The existence of positive correlations between incident light and the production of secondary compounds has been confirmed by several other studies.[284]

4.13.2 Soil factors

Other crucial environmental factors affecting plant growth are properties of the soil, including its mineral status. Numerous observations relate insect growth and abundance to the chemistry of the soil on which their host plants grow. In agriculture, application of fertilizers is generally used to promote rapid, healthy plant growth and to increase yields. Fertilization primarily influences plant physiology but can also induce changes in plant morphology and phenology. Physiological responses are manifested by changes in nutrient composition, such as protein levels. Secondary metabolism is also affected, resulting in increased or decreased levels of secondary plant substances.[102] A meta-analysis of 147 literature reports presented overwhelming evidence that high nitrogen fertilization resulted in a decrease in foliar concentrations of a range of carbon-based secondary plant compounds.[164] It should be realized that, when the increase of secondary compounds is slower than the rate of increase of biomass, their concentrations decrease.[163] In some cases the insect responds primarily to changes in the nutritive make-up of its host, whereas in other cases changes in allelochemicals appear to dominate.

Not only the nutritional status of a plant but also its leaf surface chemistry and its appearance are affected by fertilization. As a result, insects searching for a host plant to oviposit on may respond differently to fertilized plants than to unfertilized conspecifics. A spectacular sensitivity to fertilizer-induced changes has been observed in the cabbage white butterfly, *Pieris rapae*. Ovipositing females appear able to discriminate between fertilized and unfertilized host plants within 24 hours after fertilizer application.[198] This demonstrates not only that the plant may respond rapidly to the treatment, but also that insects can perceive supposedly subtle differences between their host plants.

Many insects benefit from improved plant growth and the possibly associated increase in nutritional values. However, as so often in studies of insect–plant relationships, examples can also be cited in which plant fertilization negatively affects insect populations, for example by increased vigour or by shortening the stage of susceptibility to insect attack. The fact that generalizations in this area of research are often weakened by too many exceptions, seemingly negating the rule, is not evidence of poor science but is rather due to the incredible flexibility of insects, their enormous diversity in species, and the great subtlety of the interactions between insects and plants.

As nitrogen is the element that most often limits crop productivity, nitrogen fertilization of field crops often seems to stimulate insect populations as a result of increased consumption and higher utilization rates (Fig. 4.21).[196] Scriber has reported that in about 115 studies crop damage by pest insects increased with the nitrogen content of their host plants.[236] Woody ornamental plants also show increased susceptibility to insect damage after fertilization, resulting from their enhanced nutritional quality for insects and/or decreased concentrations of secondary compounds.[134] Curiously, the opposite effects often occur after fertilizing forest trees. These differences remain to be explained.

No generalizations can be made with respect to the effects of phosphorus, potassium, or organic fertilizers on insect populations. All that can be said is that in many cases fertilization practices evidently have profound effects on insect herbivores, although ‘our present knowledge on the basic nature of soil minerals–plant–insect interactions is weak.’[60]

Environmental effects on secondary metabolism show up in some dramatic differences observed when crop plants were grown simultaneously in the field and in greenhouses. The glucosinolate content of greenhouse-grown cabbage plants reached only 10% of the levels measured in plants grown under field conditions.[52] Opposite reactions, however, were found in tomato plants. In this case the alkaloid content of greenhouse-grown plants was two to four times higher than that of plants grown in the field.[19] Lacking further information on the factors causing such significant differences, the examples given show, once again, the great influence that environmental conditions may exert

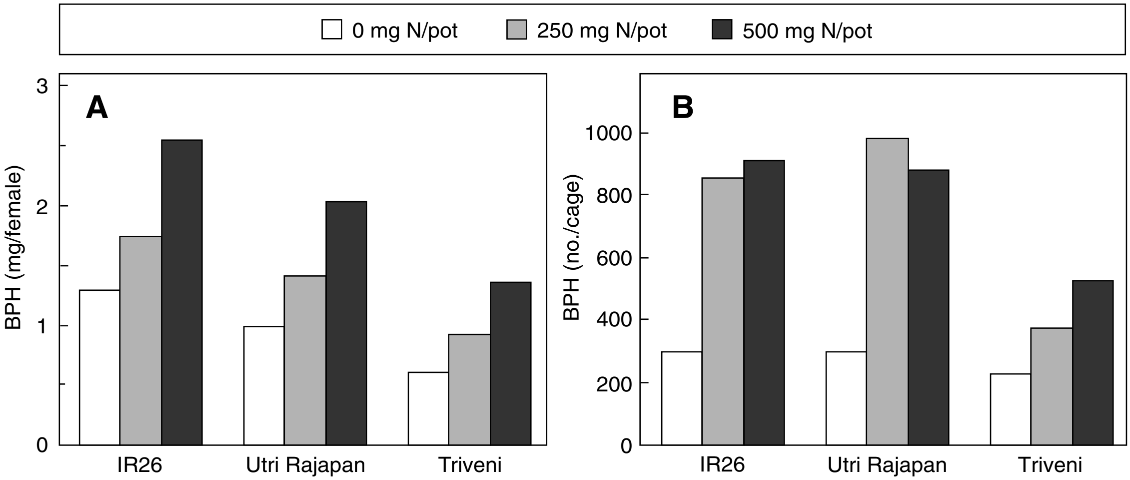


Figure 4.21 (**A**) Bodyweight of female brown plant-hoppers (*Nilaparvata lugens*) and (**B**) population development on three selected rice varieties grown in experimental cages under different nitrogen fertilization rates. IR26 is a susceptible cultivar, Utri Rajapan is tolerant and Triveni is moderately resistant. The brown plant-hopper (BPH) is one of the most devastating pest species on rice. (From Heinrichs and Medrano, 1985.)[132]

on the plant's physiology, including its allocation of resources into secondary metabolites. This cautions us against extrapolating results from greenhouse experiments on insect–plant relations to natural situations.

4.14 Induced resistance

A plant is exposed to many species of mobile attackers from which it cannot run away. However, the species of attacker that it will face is unpredictable, and therefore it may not pay to invest in defences against all attackers. Indeed, wounding can induce resistance in plants. It appears that plants can respond differently to different types of wounding and to different insect species. This is illustrated below.

The ability of plants to induce resistance in response to herbivory was discovered in the 1970s by biochemists[114] and ecologists,[127] and has subsequently captured the imagination of plant physiologists, molecular biologists, and entomologists as well. As a result, a large body of literature has accumulated (e.g. Karban and Baldwin,[151] Agrawal *et al.*,[6] Dicke and Hilker[74]). It is now widely recognized that a plant's chemical composition is not only affected by abiotic factors but may also be influenced to a considerable degree by biotic factors, such as herbivory. This is true for a wide variety of secondary metabolites, and most likely the biosynthesis of all groups of secondary metabolites may be induced by insect herbivory. There are many examples for the induction of phenolics, terpenes, alkaloids, glucosinolates, hydrocarbons, and cyanogenic glycosides.[151] In addition, enzymes and other proteins can be induced. The induced changes may affect not only herbivorous insects (*direct effects* on the inducing herbivores) but also their natural enemies such as predators and parasitoids (*indirect effects* on the inducing herbivores). As a result, four general types of plant resistance are now recognized (Table 4.7).

Induced direct resistance[151] and induced indirect resistance[71] have been reported from many plant species belonging to a range of different families, and thus appear to be common modes of plant defence. Both types of induced resistance are effective against insects with very different modes of feeding, such as biting–chewing, cell-piercing and phloem feeders, and external feeders such as caterpillars and aphids, as well as internal feeders such as leaf-miners and stem-borers.

Table 4.7 Different types of resistance of plants to herbivores

	Constitutive resistance	Inducible resistance
Direct resistance	Presence is independent of damage; affects herbivorous insects negatively	Induced by herbivory; affects herbivorous insects negatively
Indirect resistance	Presence is independent of damage; affects performance of natural enemies of herbivorous insects positively	Induced by herbivory; affects performance of natural enemies of herbivorous insects positively

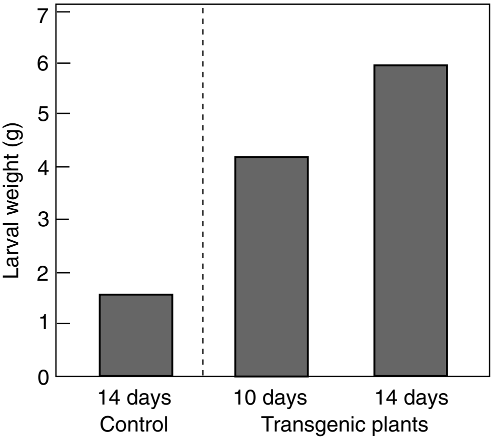


Figure 4.22 Growth of *Manduca sexta* larvae feeding on leaves of wild-type (control) and transgenic tomato plants expressing the *prosystemin* gene, which is involved in inducing resistance, in antisense orientation. (From Orozco-Cardenas *et al.*, 1993.)[207]

4.14.1 Induced direct resistance

All the mechanisms that plants employ in constitutive chemical resistance can also be activated through induction. For instance, feeding damage by the Colorado potato beetle induces potato plants to synthesize higher levels of proteinase inhibitors.[114] The proteinase inhibitors interfere with food digestion by this beetle as well as other herbivorous insects. When the plant's response is eliminated, for example by inserting an antisense copy of a gene whose product is essential in the induction process, the performance of the caterpillar *Manduca sexta* is greatly enhanced (Fig. 4.22).

The changes can also take a totally different course. Some potato genotypes respond to oviposition by Colorado potato beetles with a hypersensitive response. The plant tissue around an egg batch dies and the eggs dessicate or even fall through the leaf on to the ground, where they may be consumed by soil-dwelling predators.[14]

Clearly, induced direct resistance is widespread in the plant kingdom: it has been recorded in more than 100 plant species belonging to 34 families.[151]

4.14.2 Induced indirect resistance

Plants can also use chemicals to promote the effectiveness of natural enemies of herbivores. In response to feeding damage by spider mites (*Tetranychus urticae*), Lima bean plants emit a blend of volatiles that is qualitatively different from the blend emitted by undamaged or mechanically damaged Lima bean plants. Spider-mite feeding induces the emission of several terpenoids and methyl salicylate, and these compounds attract the rapacious predatory mite *Phytoseiulus persimilis*, which exterminates local populations of its prey (Fig. 4.23).[271]

Herbivory-induced production of carnivore-attracting volatiles has now been reported for more than 20 plant species in 13 families and there are no reports of plants that do not exhibit this response.[71] Carnivores that are attracted by herbivory-induced volatiles are not only predators but also parasitoids.[264]

The production of plant volatiles can also be induced by the deposition of insect eggs on the plant. Oviposition by the elm leaf beetle *Xanthogaleruca luteola* on elm leaves results in the emission of volatiles that attract the egg parasitoid *Oomyzus galleruca*.[187] Similarly, oviposition by the herbivorous hemipteran *Nezara viridula* induces bean plants to emit volatiles that attract the egg parasitoid *Trissolcus basalis*.[51] It is interesting to note that many characteristics of oviposition-induced plant

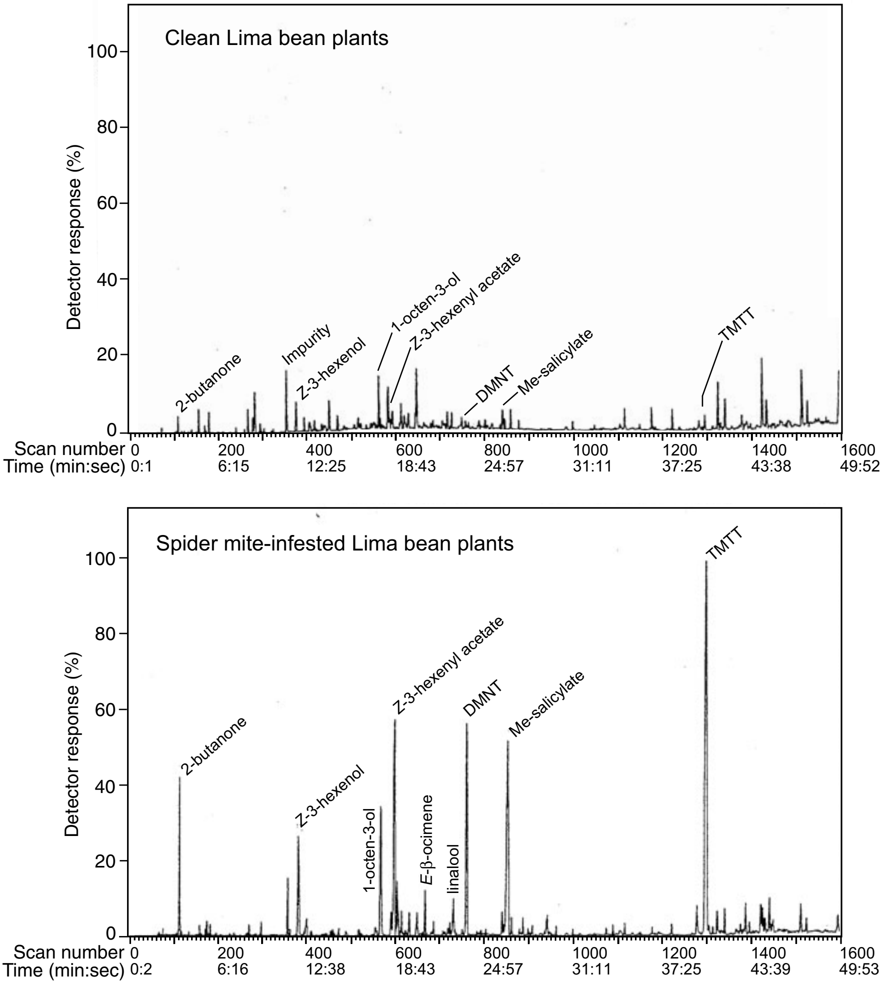


Figure 4.23 Gas chromatography–mass spectrometry analysis of volatiles emitted by clean Lima bean leaves and lima bean leaves infested with the spider mite *Tetranychus urticae*. DMNT, (*E*)-4,8-dimethyl-1,3,7-nonatriene (**18**); Me-salicylate, methyl salicylate; TMTT, (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (**69**). (M. Dicke and M.A. Posthumus, unpublished data.)

volatiles are similar to those induced by herbivory.[136] Recently, there has even been a report of induced chemical changes in plants in response to caterpillar footsteps,[34] but whether insect footsteps induce volatile emission remains to be investigated.

Mechanical damage can often not (fully) mimic herbivory or oviposition in the induction of resistance. Apparently, the response is induced by a herbivore-derived elicitor. An induced attraction of carnivorous arthropods usually occurs within one to a few days and the induced volatiles are produced not only locally but, upon stimulation via a systemically transmitted signal, also in leaves that have not been touched by the herbivore.[75,136]

Apart from an effect on parasitoids or predators of herbivores, herbivore-induced plant volatiles may also elicit behavioural responses in herbivorous insects. Some herbivores are attracted to the volatiles,[31,149] whereas other herbivores are repelled.[64,68] Thus, the emission of herbivore-induced plant volatiles may differentially affect several insect species and thereby the interactions between the plant and the associated insect community.

4.14.3 Variation in herbivore-induced changes

The chemical changes that occur in response to herbivory vary among genotypes as well as plant species. Thus, large differences in total volatile emission have been recorded among different maize genotypes (Fig. 4.24).[111] In addition, abiotic factors such as light, humidity, and nutrient availability can have significant effects. For instance, the amounts emitted by maize plants in response to mimicked caterpillar herbivory are positively correlated with the amount of daylight, and are higher at low soil humidity and high nutrient availability.[110]

The response of a plant to herbivory differs from the response to mechanical damage; even responses to different herbivore species may not be identical.[75] For instance, maize plants that are damaged by fall armyworm (*Spodoptera exigua*) caterpillars emit several terpenoids that attract the parasitoid *Cotesia marginiventris*. In contrast, mechanically damaged plants do not emit these volatiles. However, when caterpillar regurgitant is applied on to a mechanically inflicted wound, the plant's response is identical to its response to caterpillar feeding damage.[263]

There are many examples of carnivores that discriminate between the volatiles induced by different herbivore species.[70] For instance, the aphid parasitoid *Aphidius ervi* is attracted to the volatiles emitted by broad bean plants infested with their host, the pea aphid *Acyrthosiphon pisum*, whereas volatiles emitted by bean plants infested with the non-host aphid *Aphis fabae* are not attractive.[81] Apparently, subtle differences exist between the volatiles released in response to different herbivores that determine the responses of species belonging to the third trophic level. The role of individual volatiles in the discrimination of carnivores between volatiles emitted by plants infested with suitable prey and plants infested with non-prey has recently been elucidated for the predatory mite *Phytoseiulus persimilis*. Bean plants infested with the prey mite *Tetranychus urticae* emit large amounts of methyl salicylate and the terpenoid (3*E*, 7*E*)-4, 8, 12-trimethyl-1, 3, 7,11-tridecatetraene, whereas bean plants infested with the non-prey caterpillar *Spodoptera exigua* emit only trace amounts of these compounds. The predators discriminate between the blends of volatiles emitted by *Tetranychus*-infested and *Spodoptera*-infested bean plants. The role of methyl salicylate and the terpenoid (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene in this discrimination was proven by adding the individual chemicals to the volatiles emitted from *Spodoptera*-infested bean plants. This resulted in a strong increase in the attraction of the predators to *Spodoptera*-infested bean plants in a two-choice situation where *Tetranychus*-infested plants were offered as alternative.[63] This shows that different herbivores induce different blends of plant volatiles and that certain components of the blends can be used by carnivorous arthropods to discriminate.

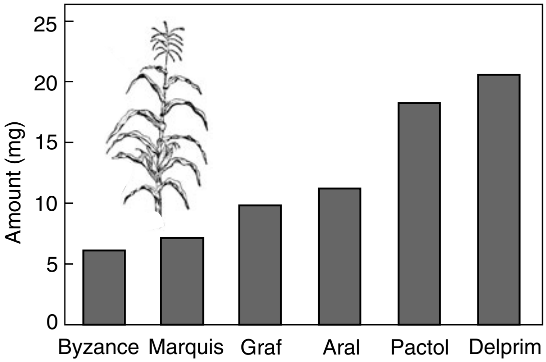


Figure 4.24 Total amount of volatiles induced by the application of regurgitant from caterpillars of *Spodoptera littoralis* on mechanical wounds in different maize varieties, collected over a 9-h period. (From Gouinguené *et al.*, 2001.)[111]

4.14.4 Genomic and metabolomic changes induced by herbivory

Herbivory can result in tremendous changes in a plant's chemical composition. For instance, a Lima bean plant that is damaged by spider mites emits approximately 30 volatile compounds, especially terpenoids, that are not released by undamaged plants.[77] Herbivory, however, results in many more physiological changes than only those related to volatile emission. Genomic analyses have shown that the expression level of many genes is modified in response to herbivory.[216] For *Nicotiana attenuata* it is estimated that the *Manduca sexta*-responsive transcriptome contains more than 500 different messenger RNAs.[135] These results indicate that the physiology of a plant changes drastically upon attack by a herbivore. Transcripts involved in photosynthesis were strongly downregulated, whereas those responding to stress, wounding, and pathogens, and those associated with the shifting of carbon and nitrogen to defence, were strongly upregulated.[135] In *Arabidopsis thaliana* the expression of 114 of 7200 genes investigated was changed in response to feeding damage by *Pieris rapae* caterpillars.[217] In a genome-wide inventory of pathogen-infected *Arabidopsis thaliana* it appeared that the expression of as many as 2375 genes was changed compared with an uninfested control. Of course, not all of these genes affect the production of plant chemicals, but many do so, directly or indirectly.

Information on the induction of genes involved in the biosynthesis of herbivore-induced plant volatiles is accumulating rapidly. Terpene synthase genes have been characterized in maize plants and differences in terpene emission among maize cultivars were shown to be caused by allelic variation within one of these genes.[162] A spider mite-inducible (*E*)-β-ocimene synthase gene has been identified in the legume *Lotus japonicus*.[12] Moreover, through a combined metabolomics and transcriptomic approach, spider mite-inducible genes involved in the biosynthesis of terpenoid volatiles have been discovered in cucumber plants.[189]

The genomic information available for *Arabidopsis thaliana* has been successfully exploited to clone many terpene synthase genes. Their expression patterns in response to different stresses may now be explored to gain an understanding of the environmental conditions that switch these volatile biosynthesis genes on and off. Many of these terpene synthase genes appear to be involved in the biosynthesis of flower volatiles, but some are involved in the biosynthesis of leaf volatiles or root compounds.[49]

The combined knowledge from different studies of a single plant–insect system supports the notion that herbivory results in extensive chemical changes. Moreover, modern technology now allows a direct investigation of such chemical changes. For instance, a metabolomics approach can analyse differences in 326 foliar chemicals from different *Arabidopsis thaliana* genotypes.[93] Further development of such methods will allow for a more comprehensive assessment of chemical changes in plants in response to herbivory. Extensive efforts from entomologists are now required to determine the biological relevance of each of these changes.

Clearly, the molecular genetic approach provides important new tools, such as mutants and transgenics that have been altered in a single gene, for determining the contribution of individual factors leading to increased resistance.[79,155,275] However, it is clear that induced resistance is rarely caused by changes in a single trait: herbivory results in large-scale transcriptome alterations. Genetic alterations of the regulatory elements that coordinate these complex changes induced by herbivory most likely present the best approach towards a functional analysis of putative defence traits.[221] Such regulatory elements may comprise, for instance, genes that regulate the expression of the octadecanoid pathway.

4.14.5 Systemic effects

In addition to local effects, a local infestation often also has systemic effects. Such distance effects relate to both induced direct and indirect resistance. Feeding damage on a single tomato leaf results in the production of protease inhibitor in other leaves of the same plant.[228] Likewise, caterpillar feeding on a single maize leaf results in the emission of parasitoid-attracting volatiles from other leaves of the same plant.[262] In some cases systemic effects are inherent even to the local effect. Nicotine synthesis in tobacco plants occurs in the roots. Caterpillar damage to tobacco leaves results in an induced nicotine production in the roots and subsequent transport to both the damaged and the undamaged leaves.[16] The exact nature of the systemic mechanism is still unsolved. There is evidence for the presence of chemical elicitors,[228] as well as a role for hydraulic[179] and electrical[292] signals. Possibly, systemic signalling is based on a combination of the three modes.

Systemic signal transmission relies on the 'plumbing' of the vascular connections of leaves.[206]

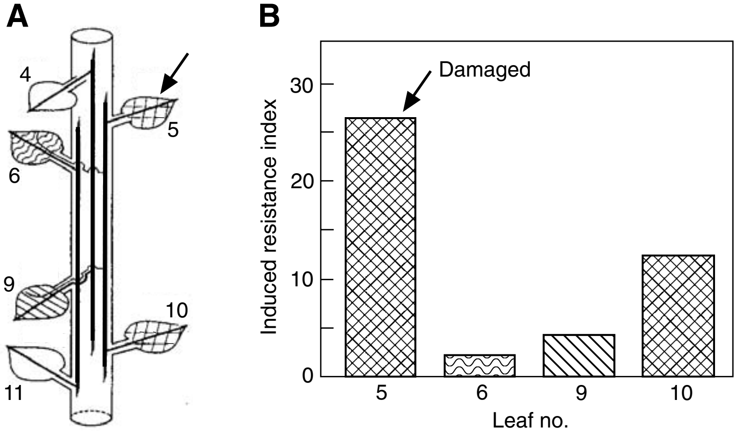


Figure 4.25 Degree of induction of resistance in Eastern cottonwood (*Populus deltoides*) by mechanical damage to a single (indicated) leaf depends on the degree of vascular connectivity between leaves. (**A**) Leaf numbers 5 and 10 share direct vascular connections, whereas leaf numbers 6 and 9 are less directly connected to leaf number 5. (**B**) The induction pattern mirrors the vascular connections between the leaves. (From Jones *et al.*, 1993.)[145]

This has been nicely illustrated in a study on cottonwood trees where every fifth leaf shares a vascular connection. As a result, the induced changes in resistance appear to be strongest in those leaves that are directly connected with the damaged leaves through their vascular system (Fig. 4.25).[145] In addition, there seems to be an upward direction in the systemic response. Lower leaves of a plant are source leaves and higher leaves are sink leaves, and the major flow of materials goes to the sink leaves. This is also reflected in systemic induced resistance. Herbivory by forest tent caterpillars (*Malacosoma disstria*) in poplar trees resulted in upregulated gene expression in higher leaves, but not in lower leaves.[11]

In conclusion, insect herbivory leads to different changes in different systemic leaves, and thus herbivory results in a spatially different phenotypic expression. This means that insect herbivores are confronted with a spatially variable resource after local feeding damage.

4.14.6 Long-term responses

The discussion so far has concerned rapid plant responses that operate on relatively short time-scales. Delayed induced responses have been found to occur later in the same season, in the next season's or later foliage of woody plants.[202] For example, early-season herbivory in the tropical plants *Croton pseudoniveus* and *Bursera instabilis* induced the production of total phenolics and condensed tannins that influenced herbivory later in the season.[29] One of the best documented examples concerns birch trees, which are abundant and widespread in the vast forests of Finland. Haukioja and his colleagues measured increased concentrations of phenolics in leaves when trees were exposed to caterpillar feeding. These changes negatively affected their nutritional value for folivores. In this case the chemical changes were manifested over two different time-scales: an increase in phenolic levels that built up in hours to days and was short-lasting, and a long-term response that lasted for months to years.[200] When larvae of *Epirrita autumnata* were reared on the foliage of trees that had been defoliated 2 or more years previously, growth and fecundity were significantly reduced (Fig. 4.26). The fact that the nutritional quality of trees after complete defoliation may affect insect performance even after several years is of ecological relevance. It introduces a time lag to the negative feedbacks regulating the population dynamics of insect herbivores

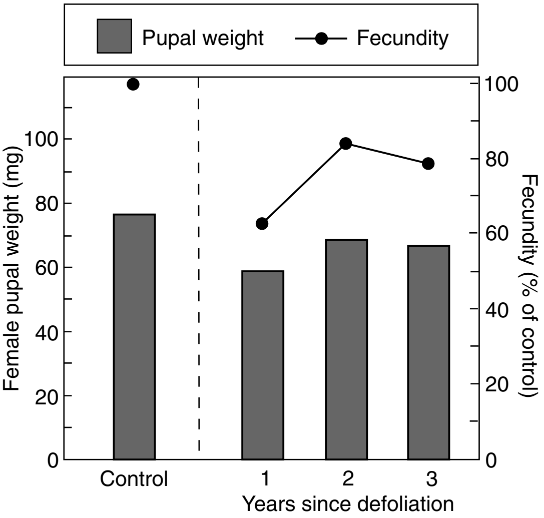


Figure 4.26 Effects of artificially defoliated mountain birch trees (*Betula pubescens*) on the growth (female pupal weight) and fecundity of the autumnal moth *Epirrita autumnata*. Insects grown on foliage picked from trees that were defoliated 2–4 years earlier still show reduced growth and fecundity. (Data from Neuvonen and Haukioja, 1991.)[200]

and may generate cyclical density fluctuations. Thus, cyclical outbreaks of the larch budmoth (*Zeiraphera diniana*) may be explained partly by chemical and morphological changes in the needles of its conifer host, *Larix decidua*, that persist for up to 4–5 years after defoliation.[21]

4.14.7 Signal transduction

There is rapid progress in our understanding of the signal transduction that leads from herbivory or wounding to induced chemical changes. Several researchers, however, have shown that phytochemical induction is stimulated more intensely and more specifically by insect secretions, indicating that 'somehow trees can distinguish between sterile scissors and caterpillar mouthparts'.[124] The differential effect of herbivore damage and mechanical damage can be explained by herbivore-derived elicitors. The effect of herbivory can be mimicked by the application of regurgitant on to mechanically wounded plant surfaces.[112,116] Several elicitors in caterpillar regurgitant have been identified, including volicitin and other fatty acid–amino acid conjugates,[116] β-glucosidase,[184] and glucose oxidase.[195]

Three main signal transduction pathways that are involved in induced responses have been distinguished in plants: (1) the octadecanoid pathway, which involves, among others, jasmonic acid; (2) the shikimate pathway, which involves mainly salicylic acid; and (3) the ethylene pathway.[76] The octadecanoid pathway appears to be the main signal transduction pathway involved in insect-induced resistance, but the other two also take part. For instance, volatile emission by Lima bean plants induced by spider-mite damage is mediated by jasmonic acid as well as by ethylene in a synergistic interaction.[137] The induced volatiles also include, among others, methyl salicylate, the methyl ester of salicylic acid. Most of the volatiles released in response to spider-mite feeding can be induced by jasmonic acid, but the production of methyl salicylate is not induced by this signal compound.[77] Whether the emission of methyl salicylate is a way of discarding salicylic acid, which is known to antagonize the effects of jasmonic acid, remains an open question. Jasmonic acid and ethylene also act synergistically in the induction of protease inhibitors in tomato,[203] but in the case of nicotine induction in wild tobacco ethylene negatively influences the effect of jasmonic acid.[147]

In other insect–plant interactions, different combinations of pathways may be activated. Thus, the octadecanoid and the salicylic acid pathway are involved in the induced attraction of the parasitoid *Cotesia rubecula* to *Pieris rapae*-infested *Arabidopsis* plants, as has been shown by using transgenic lines of the host plant.[275] Transgenic plant lines that are affected in either of the two signal transduction pathways are less attractive to the parasitoids after caterpillar feeding damage. Yet, neither of the two signal transduction pathways seems completely to regulate the induction of *Arabidopsis* volatiles.

4.14.8 Interaction between herbivore-induced and pathogen-induced changes

Plants are exposed to all kinds of attackers, including not only herbivorous insects but also pathogenic microorganisms. Plant pathogens such as bacteria and fungi induce chemical changes that are also mediated by the three main signal transduction pathways mentioned above.[210] As a result, pathogen-induced changes and herbivore-induced changes may interact through cross-talk between the signalling pathways. For instance, pathogen-induced changes in tomato plants are mediated by the salicylic acid pathway and these changes negatively influence herbivore-induced changes, which are mediated mainly by the jasmonic acid pathway.[258] The observation that pathogen-induced responses are often associated with the salicylic acid pathway, and insect-induced responses with the jasmonic acid pathway, has been reported for many plant species. Yet, recent information suggests that such a strict pathway distinction is unlikely to be present, and there are examples of pathogens that induce the jasmonic acid pathway and of insects that also induce the salicylic acid pathway.[274] When plants use different pathways in response to attack by pathogens or insects, however, this may provide herbivorous insects with an interesting option for sabotage of induced defences. Caterpillars of *Helicoverpa zea* were, surprisingly, found to induce the salicylic acid signalling pathway in soybean and not the jasmonic acid pathway. Salicylic acid-mediated changes do not result in induced resistance against these caterpillars in soybean and thus it appears that the caterpillars manipulate their food plant's signalling mechanism to suppress jasmonic acid-mediated induced resistance.[195] This example shows that the interaction of defences against multiple attackers by plants is an exciting topic awaiting further elucidation.

4.14.9 Plant–plant interactions

In the 1980s, the first evidence was found that induced chemical changes do not occur only within the damaged plant, but may also happen in neighbouring plants.[17,219] In the past decade ample evidence has accumulated to support this notion.[72,78] Evidence now exists for both induced direct and induced indirect resistance, under laboratory as well as field conditions, and for interactions among conspecific and heterospecific plants. For instance, field studies on alder trees showed that resistance was induced both in partially defoliated trees and in their untreated conspecific neighbours. Laboratory studies have unravelled the underlying mechanism, which involves the emission of volatiles from the partially defoliated trees.[260] Exposure of uninfested Lima bean leaves to volatiles emitted by spider mite-infested conspecific leaves results in the induced expression of genes involved in defence responses as well as in the induced attraction of predatory mites that are enemies of the spider mites.[10,37]

An example of heterospecific plant interaction in a field situation is provided by sagebrush. Damage to sagebrush plants results in the induction of resistance in neighbouring wild tobacco plants (*Nicotiana attenuata*). This reaction of the tobacco plants may be the physiological response to the volatile methyl jasmonate emitted by the damaged sagebrush plants.[152,212]

So far, most studies have concentrated on the role of aerial plant volatiles in the interaction between plant species. However, two studies have recently shown that above-ground herbivory can also result in below-ground exchange of chemicals that induce chemical changes in the neighbouring plants. Thus, the roots of bean plants infested with aphids or spider mites exude an elicitor that, after reaching the roots of neighbouring undamaged conspecifics, induces in these plants the emission of carnivore-attracting volatiles. Apparently, the plant is able to take preventive measures against increased risks of herbivore attack.[47,73]

The data on the effects of induced plant responses on neighbouring plants may have implications for the spatial aspects of plant quality to herbivorous insects. Adverse effects on plant quality may be present along gradients away from damaged plants.

4.15 Genotypic variation

4.15.1 Inter-individual variation in plant chemistry

Quantitative and qualitative differences in secondary compounds among individual plants are genetically controlled, although the environment exerts some modifying effects. The concentrations of most compounds are more than 50% determined by genetic variation and thus show substantial genetic influence over phenotypic variability.[24,165] Genetic control of tissue concentrations of secondary compounds can easily be demonstrated by selection experiments. In the case of *Brassica rapa* it took only three bouts of selection to produce lines with a 60% higher or 40% lower glucosinolate content than the parent population. Larvae of *Pieris rapae* and *Trichoplusia ni* consumed greater amounts of leaf area on plants from lines selected for low glucosinolate content than on high-glucosinolate plants, when given a choice.[252]

Not only are the levels of constitutive secondary metabolites under genetic control, but clearly so are those of the induced compounds synthesized upon plant damage,[54] although few studies have quantified this trait. In the case of inducible pyrrolizidine alkaloids in hound's tongue (*Cynoglossum officinale*), heritability accounted for 35% of the variation.[267] The amounts of secondary compounds occurring in plants exhibit continuous variation, and are usually polygenically controlled (in a manner involving several or many genes). Genotypic variation may be substantial in natural populations. Estimated salicin concentrations in the leaves of willow clones, for example, range from 0.05% of dry weight to over 5%, a 100-fold range, whereas the standard deviation within clones varies less than 2-fold on average.[245]

As the concentrations of secondary plant substances are generally under tight genetic control, selection may readily modify the quantities produced. As a result, striking differences are seen when the amounts of secondary chemicals in some cultivars are compared to those found in wild relatives. Of course, selection of low-allelochemical lines will change the plants' susceptibility to insect attack. Cotton (*Gossypium hirsutum*) varieties with reduced levels of gossypol (**28**), a phenolic sesquiterpene pigment, are a better food source to a number of insects than high-gossypol lines, as part of their natural resistance has been eliminated (Fig. 4.27). The production of cucurbitacin (**14**), the triterpenoid that gives cucumbers their bitter taste, is controlled by a single gene. Breeding programmes have deliberately selected non-bitter varieties to suit human taste. Because this compound is a potent deterrent and also highly toxic to many herbivores, including humans, low-cucurbitacin cultivars appear very susceptible to infestation by two-spotted spider mites (*Tetranychus urticae*) and several insect species.

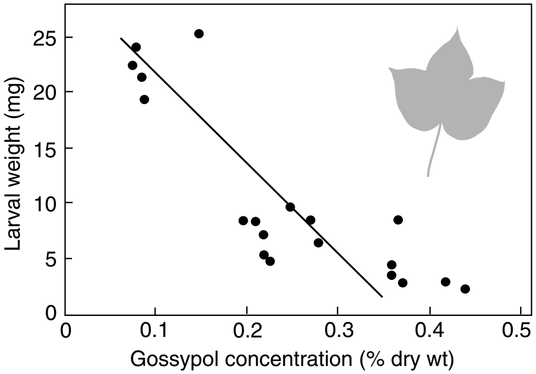


Figure 4.27 Growth of first instar tobacco budworm larvae over a 5-day period when kept on intact plants of each of 20 cotton cultivars with variable gossypol concentrations. (Data from Hedin *et al.*, 1983.)[130]

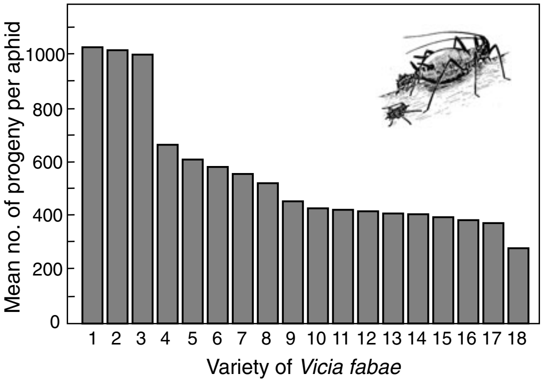


Figure 4.28 Mean number of progeny of a single aphid (*Aphis fabae*) over a 14-day period when kept on 18 different cultivars of broadbean (*Vicia faba*). (Data from Davidson, 1922.)[62]

Plant breeders employ the vast genetic variation in chemical and physical properties of natural plant species to develop cultivars with specific desirable traits. Different cultivars of almost any crop plant species appear to have different degrees of susceptibility to insect attack, because of differences in their genetic make-up (Fig. 4.28). Intense selection for fast plant growth and high reproductive output in the past generally resulted in a lowering of the plants' allocation to resistance. As a consequence, cultivated plants often became more vulnerable than their wild progenitors. Today plant breeders tend to select cultivars that still possess their natural chemical protection, except in the organs that are used as human food, such as fruits or seeds. This type of selection has been achieved with, for instance, potato tubers.[144] The topic of resistance breeding is discussed in more detail in Chapter 13.

4.15.2 Intra-individual variation in plant chemistry

Although the concept of an individual does not cause confusion with regard to most animals, including insects, it may be less clear for plants. Modular organisms make the definition of an individual more complex. Thus, a tree may be considered as a population of suborganismic units (modules), each of which develops and dies at its own time. A tree in this view can be described as a population of modules with associated stems and roots.[286] The concept of generation span then also becomes less clear. Some plant species have (in the usual sense) very long generation periods. Consequently, their rate of genetic recombination is very low compared with, for instance, insects. Broadleaf trees, which can reach an age of 200 years or more, may be considered as a monoculture in time, because of their long lifespan. Moreover, plant populations that consist essentially of clones are by nature very homogeneous and can be very long lived. Some bracken fern clones (*Pteridium aquilinum*) are supposedly 1000 years or more old. The core of the English elm (*Ulmus procera*) population in Great Britain consists, based on molecular evidence, of a 2000-year-old clone, originating from material transported by the Romans. Its genetic homogeneity would explain the rapid spread in the 1970s of Dutch elm disease, caused by a fungus that is transferred from tree to tree by elm-bark beetles (*Scolytus*).[107] Similarly, populations of some herbaceous angiosperms such as goldenrod (*Solidago missouriensis*) or woody species such as aspen trees (*Populus tremuloides*) may essentially represent one clone, which may cover large areas (several hectares) and could date back to the Pleistocene.[289] The disadvantages of the genetic rigidity of long-lived clones may be compensated by somatic mutations, which can be inherited by naturally occurring mechanisms of sexual and asexual reproduction. The accumulation of somatic mutations may permit a clone to develop as a genetically diverse individual.[87]

As a result of somatic mutations, long-lived plants may during their growth develop as mosaics in which different branches or other parts of the same plant vary greatly in their genetic composition. An example of such genetic mosaicism is provided by the susceptibility to infestation by a gall aphid, which differed markedly between different branches of an individual poplar tree.

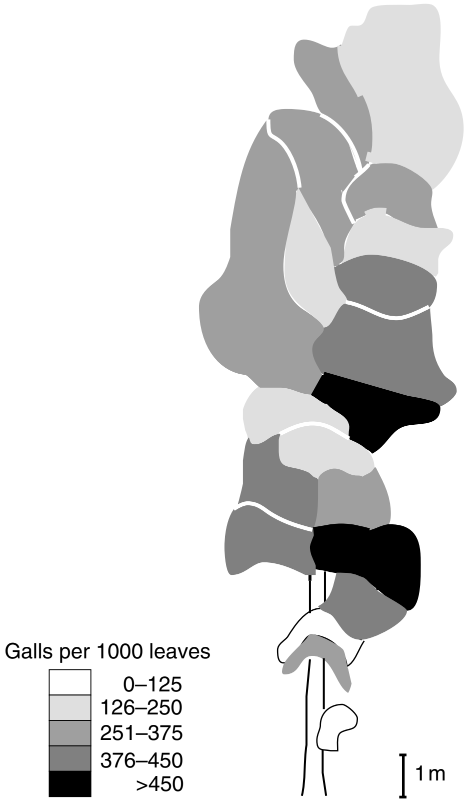


Figure 4.29 The distribution of about 53 000 *Pemphigus betae* (Aphididae) galls over 20 branches of a 20.1-m high *Populus angustifolia* tree. The size of each branch reflects total leaf area. (Redrawn from Whitham, 1983.)[289]

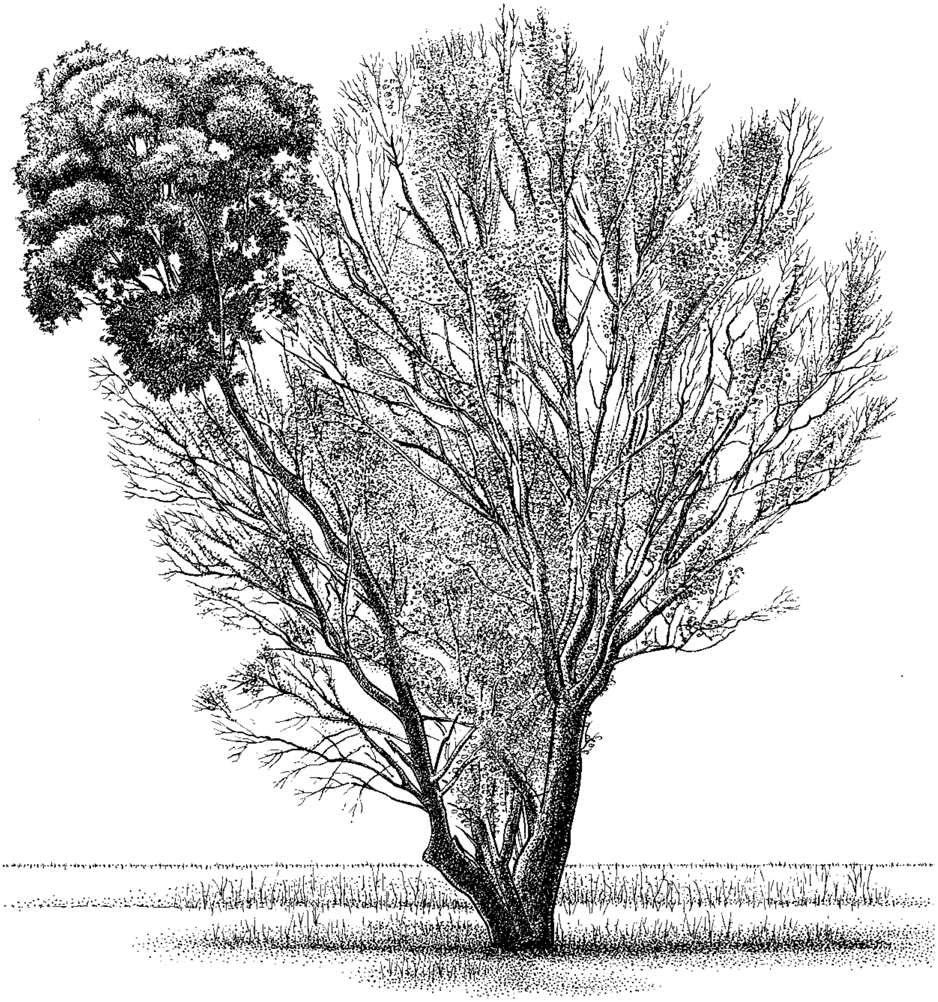


Figure 4.30 Mosaic resistance in *Eucalyptus meliodora*. The Christmas beetle *Anoplognatus montanus* may, during an outbreak, defoliate trees completely. Some branches or sometimes whole trees are, however, immune because the compositions of their volatile oils are different. The resistant plant parts most probably developed from meristematic cells containing newly arisen somatic mutations. The resistant branches will produce seeds carrying the genes for resistance (Edwards *et al.*, 1990).[87] (Drawing by P. Kostense after a photo kindly provided by P.B. Edwards.)

The distribution of galls, therefore, was not random, but reflected the underlying mosaic pattern of host resistance (Fig. 4.29). This high level of variation in susceptibility to gall aphids within an individual tree appeared to be of the same magnitude as the range of variation observed between extreme trees in the population.[289] Another example of 'mosaic resistance' is shown in Figure 4.30.

4.15.3 Plant sex affects insect susceptibility

Approximately 6% of all flowering plant species are dioecious, that is, each individual plant bears only either male or female flowers. Entomologists have noted several instances in which insects recognize plant sex differences, and often prefer ovipositing and feeding on male plants over female plants (Table 4.8). These observations raise

Table 4.8 A selection of studies reporting differential herbivory in relation to plant sex

Plant species and family	Insect species and order	Measured effects	Reference
Rumex acetosella Polygonaceae	*Conoderus vespertinus* Coleoptera	Female plants suffer greater damage	175
Salix discolor Salicaceae	*Phyllocolpa leavitti* Hymenoptera	Higher survival on female plants	97
Pistacia atlantica Anacardiaceae	*Slavum wertheimae* Homoptera	Better performance on male plants	295
Rubus chamaemorus	Four insect species	Leaves of male plants suffer greater damage	7
Salix cinerea Salicaceae	Various insect species Various orders	Male plants suffer greater damage	9
Ephedra trifurca Ephedraceae	*Lasioptera ephedrae* Diptera	More galls on male plants	27
Salix Salicaceae	Five sawfly species Hymenoptera	More insects on male plants, due to differences in leaf phenology	28
Baccharis halimifolia Asteraceae	*Trirhabda bacharidis* Coleoptera	Insects prefer feeding on male leaves, females show higher fecundity on male leaves, male leaves more tender	166

the question: does it matter? The answer is: yes, it does, because differential herbivory exerts potentially unequal selection pressures on the two sexes and, consequently, may influence the evolution of plant breeding systems. As dioecy is often associated with large plant size (e.g. trees) and wind pollination, the benefit of this reproduction strategy most likely lies in a reduced risk of inbreeding. The observation that a preponderance of insect herbivores occurs on male plants could in some cases be correlated with higher levels of nitrogen, lower levels of leaf toughness, or lower levels of defence compounds in males compared with females.[8]

4.16 Conclusions

Superimposed on a relatively uniform primary metabolism, plants produce a perplexingly wide spectrum of secondary compounds. The multifaceted roles of these chemicals are as yet poorly understood, but undoubtedly they provide protection against harsh environmental conditions, invading microorganisms, and plant-eating animals, as summarized in Figure 4.31.

When searching for general principles in nature, scientists often have to neglect slight variations that occur in measurements of all biological phenomena. This is done by averaging. In this chapter, however, emphasis has been laid on variations in the chemical composition of plants at the species level, within populations, or within an individual plant, which may be modulated by insect attack, light conditions, nutrients in soil, atmosphere, etc. These spatial and temporal variations, caused by genotypic and environmental factors, cannot be considered as deviations from the 'normal' or standard, but represent an essential feature of the strategy of plants to optimize their survival.

4.17 Literature

There is an extensive literature on secondary plant compounds. Fine introductions are provided by Vickery and Vickery[279] and Hanson.[118] The chemotaxonomy of plants is covered by Frohne and Jensen[98] and Smith.[246] The unrivalled standard work on phytochemistry is Hegnauer's *Chemotaxonomie der Pflanzen*.[131] The volumes by Rosenthal and Janzen[225] and Rosenthal and Berenbaum[224] present much information on plant compounds relevant to insects. The well known *Merck Index*[188] contains structural formulae and information on 10 000 chemicals, including plant substances and

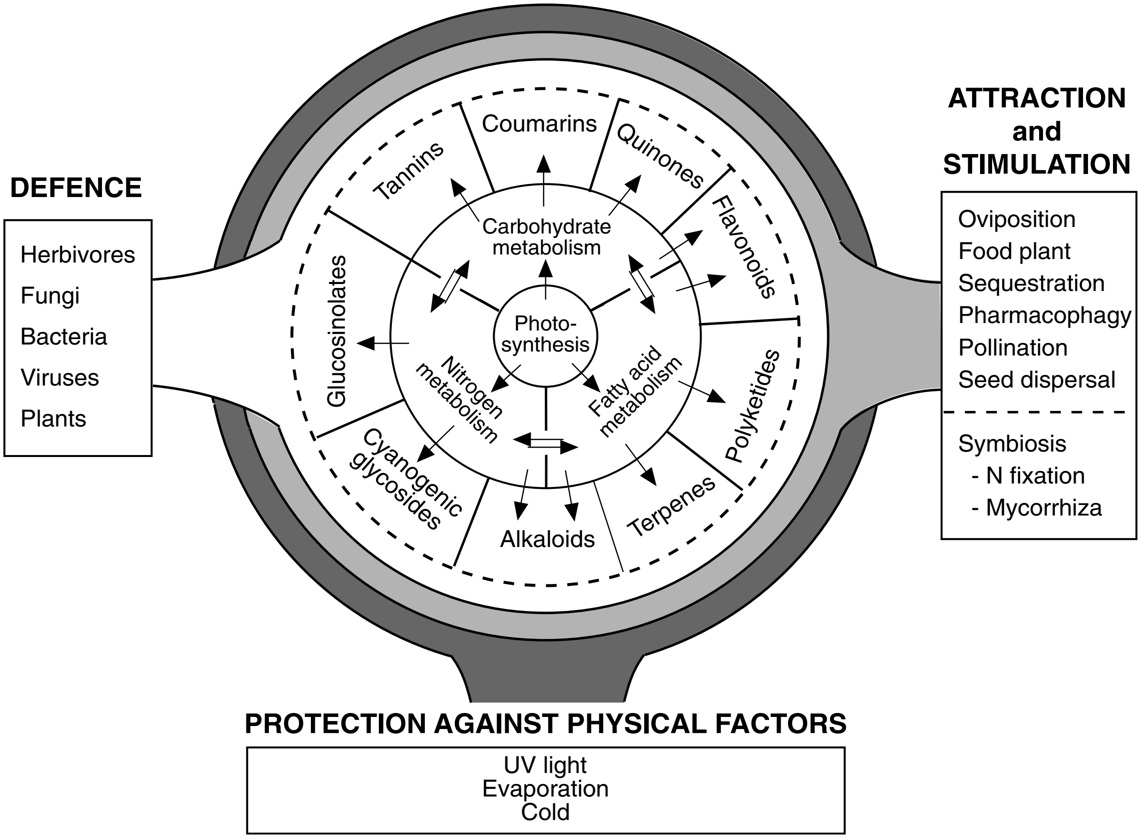


Figure 4.31 Secondary plant substances derive from primary metabolites. They show a multitude of functions and are involved in many biotic and abiotic environmental factors. (Redrawn from Hartmann, 1996.)[125]

drugs. Other recent inventories are those by Harborne and Baxter[121] and Buckingham.[39]

4.18 References

1. Abe, M. and Matsuda, K. (2000). Feeding responses of four phytophagous lady beetle species (Coleoptera: Coccinellidae) to cucurbitacins and alkaloids. *Applied Entomology and Zoology*, **35**, 257–64.
2. Abe, T. and Higashi, M. (1991). Cellulose centered perspective on terrestrial community structure. *Oikos*, **60**, 127–33.
3. Adati, T. and Matsuda, K. (1993). Feeding stimulants for various leaf beetles (Coleoptera: Chrysomelidae) in the leaf surface wax of their host plants. *Applied Entomology and Zoology*, **28**, 319–24.
4. Adel, M.M., Sehnal, F., and Jurzysta, M. (2000). Effects of alfalfa saponins on the moth *Spodoptera littorali*. *Journal of Chemical Ecology*, **26**, 1065–78.
5. Adler, L.S. and Kittelson, P.M. (2004). Variation in *Lupinus arboreus* alkaloid profiles and relationships with multiple herbivores. *Biochemical Systematics and Ecology*, **32**, 371–90.
6. Agrawal, A.A., Tuzun, S., and Bent, E. (1999). *Induced plant defenses against pathogens and herbivores*. APS Press, St Paul, MI.
7. Ågren, J. (1987). Intersexual differences in phenology and damage by herbivores and pathogens in dioecious *Rubus chamaemorus* L. *Oecologia*, **72**, 161–9.
8. Ågren, J., Danell, K., Elmqvist, T., Ericson, L., and Hjältén, J. (1999). Sexual dimorphism and biotic interactions. In *Gender and sexual dimorphism in flowering plants* (ed. M.A. Geber, T.E. Dawson, and L.F. Delph), pp. 217–46. Springer, Berlin.
9. Alliende, M.C. (1989). Demographic studies of a diocious tree. II. The distribution of leaf predation within and between trees. *Journal of Ecology*, **77**, 1048–58.

10. Arimura, G., Ozawa, R., Shimoda, T., Nishioka, T., Boland, W., and Takabayashi, J. (2000). Herbivory-induced volatiles elicit defence genes in lima bean leaves. *Nature*, **406**, 512–15.
11. Arimura, G., Huber, D.P.W., and Bohlman, J. (2004a). Forest tent caterpillars (*Malacosoma disstria*) induce local and systemic diurnal emissions of terpenoid volatiles in hybrid poplar (*Populus trichocarpa x deltoides*): cDNA cloning, functional characterization, and patterns of gene expression of (−)-germacene D synthase, PtdTPS1. *Plant Journal*, **37**, 603–16.
12. Arimura, G., Ozawa, R., Kugimiya, S., Takabayashi, J., and Bohlmann, J. (2004b). Herbivore-induced defense response in a model legume. Two-spotted spider mites induce emission of (*E*)-β-ocimene and transcript accumulation of (*E*)-β-ocimene synthase in *Lotus japonicus*. *Plant Physiology*, **135**, 1976–83.
13. Ashihara, H. and Crozier, A. (2001). Caffeine: a well known but little mentioned compound in plant science. *Trends in Plant Science*, **6**, 407–13.
14. Balbyshev, N.F. and Lorenzen, J.H. (1997). Hypersensitivity and egg drop, a novel mechanism of host-plant resistance to Colorado potato beetle (Coleoptera: Chrysomelidae). *Journal of Economic Entomology*, **90**, 652–7.
15. Baldwin, I.T. (1994). Chemical changes rapidly induced by folivory. In *Insect-plant interactions*, Vol. 5 (ed. E.A. Bernays), pp. 1–23. CRC Press, Boca Raton.
16. Baldwin, I.T. (1999) Inducible nicotine production in native *Nicotiana* as an example of adaptive phenotypic plasticity. *Journal of Chemical Ecology*, **25**, 3–30.
17. Baldwin, I.T. and Schultz, J.C. (1983). Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants. *Science*, **221**, 277–9.
18. Balkema-Boomstra, A.G., Zijlstra, S., Verstappen, F.W.A., Inggamer, H., Mercke, P.E., Jongsma, M.A., *et al.* (2003). Role of cucurbitacin c in resistance to spider mite (*Tetranychus urticae*) in cucumber (*Cucumis sativus* L.). *Journal of Chemical Ecology*, **29**, 225–35.
19. Barbour, J.D. and Kennedy, G.G. (1991). Role of steroidal glycoalkaloid α-tomatine in host-plant resistance of tomato to Colorado potato beetle. *Journal of Chemical Ecology*, **17**, 989–1005.
20. Bell, E.A. and Janzen, D.H. (1971). Medical and ecological considerations on L-dopa and 5-HTP in seeds. *Nature*, **229**, 136–7.
21. Benz, G. (1974). Negative Rückkoppelung durch Raum- und Nahrungskonkurrenz sowie zyklische Veränderungen der Nahrungsgrundlage als Regelprinzip in der Populationsdynamik des Grauen Lärchenwicklers, *Zeiraphera diniana* (Guenee) (Lep., Tortricidae). *Zeitschrift für angewandte Entomologie*, **76**, 196–228.
22. Berenbaum, M.R. (1995). Turnabout is fair play: secondary roles for primary compounds. *Journal of Chemical Ecology*, **21**, 925–40.
23. Berenbaum, M. and Seigler, D. (1992). Biochemicals: engineering problems for natural selection. In *Insect chemical ecology* (ed. B.D. Roitberg and M.B. Isman), pp. 89–121. Chapman & Hall, New York.
24. Berenbaum, M. and Zangerl, A.R. (1992). Genetics of secondary metabolism and herbivore resistance in plants. In *Herbivores. Their interactions with secondary plant metabolites*, Vol. II (2nd edn) (ed. G.A. Rosenthal and M.R. Berenbaum), pp. 415–38. Academic Press, San Diego.
25. Bingaman, B.R. and Hart, E.R. (1993). Clonal and leaf age variation in *Populus* phenolic glycosides: implications for host selection by *Chrysomela scripta* (Coleoptera: Chrysomelidae). *Environmental Entomology*, **22**, 397–403.
26. Blaakmeer, A., Geervliet, J.B.F., van Loon, J.J.A., Posthumus, M.A., van Beek, T.A., and de Groot, Æ. (1994). Comparative headspace analysis of cabbage plants damaged by two species of *Pieris* caterpillars: consequences for in-flight host location by *Cotesia* parasitoids. *Entomologia Experimentalis et Applicata*, **73**, 175–82.
27. Boecklen, W.J. and Hoffman, M.T. (1993). Sex-biased herbivory: the importance of sex-by-environment interactions. *Oecologia*, **96**, 49–55.
28. Boecklen, W.J., Price, P.W., and Mopper, S. (1990). Sex and drugs and herbivores: sex-biased herbivory in arroyo willow (*Salix lasiolepis*). *Ecology*, **71**, 581–8.
29. Boege, K. (2004). Induced responses in three tropical dry forest plant species—direct and indirect effects on herbivory. *Oikos*, **107**, 541–8.
30. Boland, W., Feng, Z., Donath, J., and Gäbler, A. (1992). Are acyclic C11 and C16 homoterpenes plant volatiles indicating herbivory? *Naturwisschenschaften*, **79**, 368–71.
31. Bolter, C.J., Dicke, M., Van Loon, J.J.A., Visser, J.H., and Posthumus, M.A. (1997). Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *Journal of Chemical Ecology*, **23**, 1003–23.
32. Boufford, D.E., Kjaer, A., Madsen, J.O., and Skrydstrup, T. (1989). Glucosinolates in Bretschneideraceae. *Biochemical and Systematic Ecology*, **17**, 375–9.
33. Bowers, M.D. (2003). Hostplant suitability and defensive chemistry of the catalpa sphinx, *Ceratomia catalpae*. *Journal of Chemical Ecology*, **29**, 2359–67.

34. Bown, A.W., Hall, D.E., and MacGregor, K.B. (2002). Insect footsteps on leaves stimulate the accumulation of 4-aminobutyrate and can be visualized through increased chlorophyll fluorescence and superoxide production. *Plant Physiology*, **129**, 1430–4.
35. Broadway, R.M. and Colvin, A.A. (1992). Influence of cabbage proteinase inhibitors *in situ* on the growth of larval *Trichoplusia ni* and *Pieris rapae*. *Journal of Chemical Ecology*, **18**, 1009–24.
36. Brooks, J.S. and Feeny, P. (2004). Seasonal variation in *Daucus carota* leaf-surface and leaf-tissue chemical profiles. *Biochemical Systematics and Ecology*, **32**, 769–82.
37. Bruin, J., Sabelis, M.W., and Dicke, M. (1995). Do plants tap SOS signals from their infested neighbours? *Trends in Ecology and Evolution*, **10**, 167–70.
38. Buchanan, B.B., Gruissen, W., and Jones, R.L. (2000). *Biochemistry and molecular biology of plants*. American Society of Plant Physiologists, Rockville, MD.
39. Buckingham, J. (1993). *Dictionary of natural products*, Vols. 1–8. Chapman & Hall, London.
40. Burns, A.E., Gleadow, R.M., and Woodrow, I.E. (2002). Light alters the allocation of nitrogen to cyanogenic glycosides in *Eucalyptus cladocalyx*. *Oecologia*, **133**, 288–94.
41. Buttery, R.G. and Ling, L.C. (1984). Corn leaf volatiles: identification using Tenax trapping for possible insect attractants. *Journal of Agricultural and Food Chemistry*, **32**, 1104–6.
42. Caballero, C., Castañera, P., Ortego, F., Fontana G., Pierro, P., Savona, G., *et al.* (2001). Effects of ajugarins and related neoclerodane diterpenoids on feeding behaviour of *Leptinotarsa decemlineata* and *Spodoptera exigua* larvae. *Phytochemistry*, **58**, 249–56.
43. Callaway, R.M. (2002). The detection of neighbors by plants. *Trends in Ecology and Evolution*, **17**, 104–5.
44. Castro, A.M., Rumi, C.P., and Arriaga, H.O. (1988). Influence of greenbug on root growth of resistant and susceptible barley genotypes. *Environmental and Experimental Botany*, **28**, 61–72.
45. Cavalier-Smith, T. (1992). Origins of secondary metabolism. In *Secondary metabolites: their function and evolution* (ed. D.J. Chadwick and J. Whelan), pp. 64–87. John Wiley, Chichester.
46. Cervantes, D.E., Eigenbrode, S.D., Ding, H.J., and Bosque-Pérez, N.A. (2002). Oviposition responses by Hessian fly, *Mayetiola destructor*, to wheats varying in surface waxes. *Journal of Chemical Ecology*, **28**, 193–210.
47. Chamberlain, K., Guerrieri, E., Pennacchio, F., Pettersson, J., Pickett, J.A., Poppy, G.M., *et al.* (2001). Can aphid-induced plant signals be transmitted aerially and through the rhizosphere? *Biochemical Systematics and Ecology*, **29**, 1063–74.
48. Chapman, R.F. and Bernays, E.A. (1989). Insect behavior at the leaf surface and learning as aspects of host plant selection. *Experientia*, **45**, 215–22.
49. Chen, F., Tholl, D., D'Auria, J.C., Farooq, A., Pichersky, E., and Gershenzon, J. (2003). Biosynthesis and emission of terpenoid volatiles from *Arabidopsis* flowers. *Plant Cell*, **15**, 481–94.
50. Chew, F.S. and Renwick, J.J.A. (1995). Host-plant choice in *Pieris* butterflies. In *Chemical ecology of insects*, Vol. 2. (ed. R.T. Cardé and W.J. Bell), pp. 214–38. Chapman & Hall, New York.
51. Colazza, S., Fucarino, A., Peri, E., Salerno, G., Conti, E., and Bin, F. (2004). Insect oviposition induces volatile emission in herbaceous plants that attracts egg parasitoids. *Journal of Experimental Biology*, **207**, 47–53.
52. Cole, R.A. (1994). Locating a resistance mechanism to the cabbage aphid in two wild Brassicas. *Entomologia Experimentalis et Applicata*, **71**, 23–31.
53. Cole, R.A. (1997). The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated brassica species. *Entomologia Experimentalis et Applicata*, **85**, 121–33.
54. Coleman, J.S. and Jones, C.G. (1991). A phytocentric perspective of phytochemical induction by herbivores. In *Phytochemical induction by herbivores* (ed. D.W. Tallamy and M.J. Raupp), pp. 3–45. John Wiley, New York.
55. Coley, P.D. (1986). Costs and benefits of defense by tannins in a neotropical tree. *Oecologia*, **70**, 238–41.
56. Coley, P.D., Bryant, J.P., and Chapin, T. (1985). Resource availability and plant antiherbivore defense. *Science*, **230**, 895–9.
57. Collinge, S.K. and Louda, S.M. (1988). Herbivory by leaf miners in response to experimental shading of a native crucifer. *Oecologia*, **75**, 559–66.
58. Cooper-Driver, G., Finch, S., and Swain, T. (1977). Seasonal variation in secondary plant compounds in relation to palatability of *Pteridium aquilinum*. *Biochemical and Systematic Evolution*, **5**, 177–83.
59. Costa, J.T., Gotzek, D.A., and Janzen, D.H. (2003). Late-instar shift in foraging strategy and trail pheromone use by caterpillars of the neotropical moth *Arsenura armida* (Cramer) (Saturniidae: Arsenurinae). *Journal of the Lepidopterists' Society*, **57**, 220–9.

60. Dale, D. (1988). Plant-mediated effects of soil mineral stresses on insects. In *Plant stress–insect interactions* (ed. E.A. Heinrichs), pp. 35–110. John Wiley, New York.
61. Daniewski, W.M., Gumulka, M., Anczewski, W., Masnyk, M., Bloszyk, E., and Gupta, K.K. (1998). Why the yew tree (*Taxus baccata*) is not attacked by insects. *Phytochemistry*, **49**, 1279–82.
62. Davidson, J. (1922). Biological studies of *Aphis rumicis* Linn. Reproduction on varieties of *Vicia faba. Annals of Applied Biology*, **9**, 135–42.
63. De Boer, J.G., Posthumus, M.A., and Dicke, M. (2004). Identification of volatiles that are used in discrimination between plants infested with prey or non-prey herbivores by a predatory mite. *Journal of Chemical Ecology*, **30**, 2215–30.
64. De Moraes, C.M., Mescher, M.C., and Tumlinson, J.H. (2001). Caterpillar-induced nocturnal plant volatiles repel nonspecific females. *Nature*, **410**, 577–80.
65. Denno, R.F. and McClure, M.S. (1983). *Variable plants and herbivores in natural and managed systems*. Academic Press, San Diego.
66. Derridj, S. (1996). Nutrients on the leaf surface. In *Aerial plant surface microbiology* (ed. C.E. Morris, P.C. Nicot, and C. Nguyen-The), pp. 25–42. Plenum Press, New York.
67. Derridj, S., Wu, B.R., Stammitti, L., Garrec, J.P., and Derrien, A. (1996). Chemicals on the leaf surface, information about the plant available to insects. *Entomologia Experimentalis et Applicata*, **80**, 197–201.
68. Dicke, M. (1986). Volatile spider-mite pheromone and host-plant kairomone, involved in spaced-out gregariousness in the spider mite *Tetranychus urticae. Physiological Entomology*, **11**, 251–62.
69. Dicke, M. (1994). Local and systemic production of volatile herbivore-induced terpenoids: their role in plant–carnivore mutualism. *Journal of Plant Physiology*, **143**, 465–72.
70. Dicke, M. (1999a). Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods? *Entomologia Experimentalis et Applicata*, **92**, 131–42.
71. Dicke, M. (1999b). Evolution of induced indirect defence of plants. In *The ecology and evolution of inducible defenses* (ed. R. Tollrian and C.D. Harvell), pp. 62–88. Princeton University Press, Princeton, NJ.
72. Dicke, M. and Bruin, J. (2001). Chemical information transfer between plants: back to the future. *Biochemical Systematics and Ecology*, **29**, 981–94.
73. Dicke, M. and Dijkman, H. (2001). Within-plant circulation of systemic elicitor of induced defence and release from roots of elicitor that affects neighbouring plants. *Biochemical Systematics and Ecology*, **29**, 1075–87.
74. Dicke, M. and Hilker, M. (2003). Induced plant defences: from molecular biology to evolutionary ecology. *Basic and Applied Ecology*, **4**, 3–14.
75. Dicke, M. and Van Loon, J.J.A. (2000). Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata*, **97**, 237–49.
76. Dicke, M. and Van Poecke, R.M.P. (2002). Signalling in plant–insect interactions: signal transduction in direct and indirect plant defence. In *Plant signal transduction* (ed. D. Scheel and C. Wasternack), pp. 289–316. Oxford University Press, Oxford.
77. Dicke, M., Gols, R., Ludeking, D., and Posthumus, M.A. (1999). Jasmonic acid and herbivory differentially induce carnivore-attracting plant volatiles in lima bean plants. *Journal of Chemical Ecology*, **25**, 1907–22.
78. Dicke, M., Agrawal, A.A., and Bruin, J. (2003). Plants talk, but are they deaf? *Trends in Plant Science*, **8**, 403–5.
79. Dicke, M., Van Loon, J.J.A., and De Jong, P.W. (2004). Ecogenomics benefits community ecology. *Science*, **305**, 618–19.
80. Dinan, L. (2001). Phytoecdysteroids: biological aspects. *Phytochemistry*, **57**, 325–39.
81. Du, Y., Poppy, G.M., Powell, W., Pickett, J.A., Wadhams, L.J., and Woodcock, C.M. (1998). Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi. Journal of Chemical Ecology*, **24**, 1355–68.
82. Dudareva, N., Pichersky, E., and Gershenzon, J. (2004). Biochemistry of plant volatiles. *Plant Physiology*, **135**, 1893–902.
83. Dudt, J.F. and Shure, D.J. (1994). The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology*, **75**, 86–98.
84. Dussourd, D.E. (1995). Entrapment of aphids and whiteflies in lettuce latex. *Annals of the Entomological Society of America*, **88**, 163–72.
85. Dussourd, D.E. and Hoyle, A.M. (2000). Poisoned plusiines: toxicity of milkweed latex and cardenolides to some generalist caterpillars. *Chemoecology*, **10**, 11–16.
86. Dyer, L.A. and Coley, P.D. (2002). Tritrophic interactions in tropical versus temperate communities. In *Multitrophic level interactions* (ed. T. Tscharntke and B.A. Hawkins), pp. 67–88. Cambridge University Press, Cambridge.
87. Edwards, P.B., Wanjura, W.J., Brown, W.V., and Dearn, J.M. (1990). Mosaic resistance in plants. *Nature*, **347**, 434.
88. Eigenbrode, S.D. and Espelie, K.E. (1995). Effects of plant epicuticular lipids on insect herbivores. *Annual Review of Entomology*, **40**, 171–94.

89. Facchini, P.J. (2001). Alkaloid biosynthesis in plants: biochemistry, cell biology, molecular regulation, and metabolic engineering applications. *Annual Review of Plant Physiology*, **52**, 29–66.
90. Fahey, J.W., Zalcmann, A.T., and Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, **56**, 5–51.
91. Feeny, P. (1970). Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology*, **51**, 565–81.
92. Fellows, L.E., Kite, G.C., Nash, R.J., Simmonds, M.S.J., and Schofield, A.M. (1989). Castanospermine, swainsonine and related polyhydroxy alkaloids: structure, distribution and biological activity. *Recent Advances in Phytochemistry*, **23**, 395–427.
93. Fiehn, O. (2002). Metabolomics—the link between genotypes and phenotypes. *Plant Molecular Biology*, **48**, 155–71.
94. Forkner, R.E., Marquis, R.J., and Lill, J.T. (2004). Feeny revisited: condensed tannins as anti-herbivore defences in leaf-chewing herbivore communities of *Quercus*. *Ecological Entomology*, **29**,174–87.
95. Fraenkel, G.S. (1959). The raison d'être of secondary plant substances. *Science*, **129**, 1466–70.
96. Fraenkel, G.S. (1969). Evaluation of our thoughts on secondary plant substances. *Entomologia Experimentalis et Applicata*, **12**, 473–86.
97. Fritz, R.S, Crabb, B.A., and Hochwender, C.G. (2003). Preference and performance of a gall-inducing sawfly: plant vigor, sex, gall traits and phenology. *Oikos*, **102**, 601–13.
98. Frohne, D. and Jensen, U. (1973). *Systematik des Pflanzenreichs*. G. Fischer, Stuttgart.
99. Fung, S.Y. and Herrebout, W.M. (1988). Sorbitol and dulcitol in celastraceous and rosaceous plants, hosts of *Yponomeuta* spp. *Biochemical and Systematic Ecology*, **16**, 191–4.
100. Gall, L.F. (1987). Leaflet position influences caterpillar feeding and development. *Oikos*, **49**, 172–6.
101. Geiger, D.R. and Servaites, J.C. (1994). Diurnal regulation of photosynthetic carbon metabolism in C_3 plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **45**, 235–56.
102. Gershenzon, J. (1984). Changes in the levels of plant secondary metabolites and water and nutrient stress. *Recent Advances in Phytochemistry*, **18**, 273–320.
103. Gershenzon, J. (1994a). Metabolic costs of terpenoid accumulation in higher plants. *Journal of Chemical Ecology*, **20**, 1281–354.
104. Gershenzon, J. (1994b). The cost of plant chemical defense against herbivory: a biochemical perspective. In *Insect–plant interactions*, Vol. 5 (ed. E.A. Bernays), pp. 105–73. CRC Press, Boca Raton.
105. Gershenzon, J. and Croteau, R. (1991). Terpenoids. In *Herbivores. Their interactions with secondary plant metabolites*, Vol. 1 (2nd edn) (ed. G.A. Rosenthal and M.R. Berenbaum), pp. 165–219. Academic Press, San Diego.
106. Gershenzon, J., McConkey, M.E., and Croteau, R.B. (2000). Regulation of monoterpene accumulation in leaves of peppermint. *Plant Physiology*, **122**, 205–13.
107. Gil, L., Fuentes-Utrilla, P., Soto, Á., Cervera, M.T., and Collada, C. (2004). English elm is a 2000-year-old Roman clone. *Nature*, **431**, 1053.
108. Gleadow, R.M. and Woodrow, I.E. (2002). Constraints on effectiveness of cyanogenic glycosides in herbivore defense. *Journal of Chemical Ecology*, **28**, 1301–13.
109. Gómez, D., Azorín, J., Bastida, J., Viladomat, F., and Codina, C. (2003). Seasonal and spatial variations of alkaloids in *Merendera montana* in relation to chemical defense and phenology. *Journal of Chemical Ecology*, **29**, 1117–26.
110. Gouinguené, S.P. and Turlings, T.C.J. (2002). The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology*, **129**, 1296–307.
111. Gouinguené, S., Degen, T., and Turlings, T.C.J. (2001). Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology*, **11**, 9–16.
112. Gouinguené, S., Alborn, H., and Turlings, T.C.J. (2003). Induction of volatile emissions in maize by different larval instars of *Spodoptera littoralis*. *Journal of Chemical Ecology*, **29**, 145–62.
113. Gould, K.S. and Lee, D.W. (ed) (2002). Anthocyanins in leaves. *Advances in Botanical Research*, **37**, 1–212.
114. Green, T.R. and Ryan, C.A. (1972). Wound induced proteinase inhibitors in plant leaves. *Science*, **175**, 776–7.
115. Gref, R. and Tenow, O. (1987). Resin acid variation in sun and shade needles of Scots pine (*Pinus sylvestris*). *Canadian Journal of Forest Research*, **17**, 346–9.
116. Halitschke, R., Schittko, U., Pohnert, G., Boland, W., and Baldwin, I.T. (2001). Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. III. Fatty acid–amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. *Plant Physiology*, **125**, 711–17.

117. Han, K. and Lincoln, D.E. (1994). The evolution of carbon allocation to plant secondary metabolites: a genetic analysis of cost in *Diplacus aurantiacus. Evolution*, **48**, 1550–63.
118. Hanson, J.R. (2003). *Natural products: the secondary metabolites*. Royal Society of Chemistry, Cambridge, UK.
119. Harborne, J.B. (1993). *Introduction to ecological biochemistry* (4th edn). Academic Press, London.
120. Harborne, J.B. (1994). Phenolics. In *Natural products. Their chemistry and biological significance* (ed. J. Mann, R.S. Davidson, J.B. Hobbs, D.V. Banthorpe, and J.B. Harborne), pp. 362–88. Longman, Harlow.
121. Harborne, J.B. and Baxter, H. (1993). *Phytochemical dictionary. A handbook of bioactive compounds from plants*. Taylor & Francis, London.
122. Hare, J.D. (2002). Seasonal variation in the leaf resin components of *Mimulus aurantiacus. Biochemical Systematics and Ecology*, **30**, 709–20.
123. Harley, P.C., Monson, R.K., and Lerdau, M.T. (1999). Ecological and evolutionary aspects of isoprene emission from plants. *Oecologia*, **118**, 109–23.
124. Hartley, S.E. and Lawton, J.H. (1991). Biochemical aspects and significance of the rapidly induced accumulation of phenolics in birch foliage. In *Phytochemical induction by herbivores* (ed. D.W. Tallamy and M.J. Raupp), pp. 105–32. John Wiley, New York.
125. Hartmann, T. (1996). Diversity and variability of plant secondary metabolism: a mechanistic view. *Entomologia Experimentalis et Applicata*, **80**, 177–88.
126. Hatanaka, A. (1993). The biogeneration of green odour by green leaves. *Phytochemistry*, **34**, 1201–18.
127. Haukioja, E. and Hakala, T. (1975). Herbivore cycles and periodic outbreaks. Formulation of a general hypothesis. *Report of the Kevo Subarctic Research Station*, **12**, 1–9.
128. Haukioja, E., Ossipov, V., and Lempa, K. (2002). Interactive effects of leaf maturation and phenolics on consumption and growth of a geometrid moth. *Entomologia Experimentalis et Applicata*, **104**, 125–36.
129. Hedin, P.A., Thompson, A.C., and Gueldner, R.C. (1975). Survey of the air space volatiles of the cotton plant. *Phytochemistry*, **14**, 2088–90.
130. Hedin, P.A., Jenkins, J.N., Collum D.H., White, W.H., and Parrott, W.L. (1983). Multiple factors in cotton contributing to resistance to the tobacco budworm, *Heliothis virescens* F. In *Plant resistance to insects* (ed. P.A. Hedin), pp. 347–65. American Chemical Society Symposium 208, Washington, DC.
131. Hegnauer, R. (1962–2001). *Chemotaxonomie der Pflanzen*, Vols 1–11. Birkhäuser, Basel.
132. Heinrichs, E.A. and Medrano, F.G. (1985). Influence of N fertilizer on the population development of brown planthopper (BPH). *International Rice Research Newsletter*, **10**(6), 20–1.
133. Henriksson, J., Haukioja, E., Ossipov, V., Ossipova, S., Sillanpää, S., Kapari, L., *et al.* (2003). Effects of host shading on consumption and growth of the geometrid *Epirrita autumnata*: interactive roles of water, primary and secondary compounds. *Oikos*, **103**, 3–16.
134. Herms, D.A. (2002). Effects of fertilization on insect resistance of woody ornamental plants: reassessing an entrenched paradigm. *Environmental Entomology*, **31**, 923–33.
135. Hermsmeier, D., Schittko, U., and Baldwin, I.T. (2001). Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. I. Large-scale changes in the accumulation of growth- and defense-related plant mRNAs. *Plant Physiology*, **125**, 683–700.
136. Hilker, M. and Meiners, T. (2002). Induction of plant responses to oviposition and feeding by herbivorous arthropods: a comparison. *Entomologia Experimentalis et Applicata*, **104**, 181–92.
137. Horiuchi, J., Arimura, G., Ozawa, R., Shimoda, T., Takabayashi, J., and Nishioka, T. (2001). Exogenous ACC enhances volatiles production mediated by jasmonic acid in lima bean leaves. *FEBS Letters*, **509**, 332–6.
138. Hruska, A.J. (1988). Cyanogenic glucosides as defense compounds: a review of the evidence. *Journal of Chemical Ecology*, **14**, 2213–17.
139. Ikonen, A. (2002). Preferences of six leaf beetle species among qualitatively different leaf age classes of three Salicaceous host species. *Chemoecology*, **12**, 23–8.
140. Jetter, R. and Riederer, M. (1996). Cuticular waxes from the leaves and fruit capsules of eight Papaveraceae species. *Canandian Journal of Botany*, **74**, 419–30.
141. Jetter, R. and Riederer, M. (2000). Composition of cuticular waxes on *Osmunda regalis* fronds. *Journal of Chemical Ecology*, **26**, 399–412.
142. Jetter, R. and Schäffer, S. (2001). Chemical composition of the *Prunus laurocerasus* leaf surface. Dynamic changes of the epicuticular wax film during leaf development. *Plant Physiology*, **126**, 1725–35.
143. Jetter, R., Schäffer, S., and Riederer, M. (2000). Leaf cuticular waxes are arranged in chemically and

mechanically distinct layers: evidence from *Prunus laurocerasus*. *Plant, Cell and Environment*, **23**, 619–28.

144. Johns, T. and Alonso, J.G. (1990). Glycoalkaloid change during domestication of the potato, S*olanum* section *Petota*. *Euphytica*, **50S**, 203–10.
145. Jones, C.G., Hopper, R.F., Coleman, J.S., and Krischik, V.A. (1993). Control of systemically induced herbivore resistance by plant vascular architecture. *Oecologia*, **93**, 452–6.
146. Juniper, B.E. and Southwood T.R.E. (1986). *Insects and the plant surface*. Edward Arnold, London.
147. Kahl, J., Siemens, D.H., Aerts, R.J., Gäbler, R., Kühnemann, F., Preston, C.A., *et al*. (2000). Herbivore-induced ethylene suppresses a direct defense but not a putative indirect defense against an adapted herbivore. *Planta*, **210**, 336–42.
148. Kainulainen, P., Nissinen, A., Piirainen, A., Tiilikkala, K., and Holopainen, J.K. (2002). Essential oil composition in leaves of carrot varieties and preference of specialist and generalist sucking insect herbivores. *Agricultural and Forest Entomology*, **4**, 211–16.
149. Kalberer, N.M., Turlings, T.C.J., and Rahier, M. (2001). Attraction of a leaf beetle (*Oreina cacaliae*) to damaged host plants. *Journal of Chemical Ecology*, **27**, 647–61.
150. Karban, R. (1992). Plant variation: its effect on populations of herbivorous insects. In *Plant resistance to herbivores and pathogens* (ed. R.S. Fritz and E.L. Simms), pp. 195–215. University of Chicago Press, Chicago.
151. Karban, R. and Baldwin, I.T. (1997). *Induced responses to herbivory*. Chicago University Press, Chicago.
152. Karban, R., Baldwin, I.T., Baxter, K.J., Laue, G., and Felton, G.W. (2000). Communication between plants: induced resistance in wild tobacco plants following clipping of neighboring sagebrush. *Oecologia*, **125**, 66–71.
153. Karowe, D. (1989). Facultative monophagy as a consequence of prior feeding experience: behavioural and physiological specialization in *Colias philodice* larvae. *Oecologia*, **78**, 106–11.
154. Kellogg, D.W., Taylor, T.N., and Krings, M. (2002). Effectiveness in defense against phytophagous arthropods of the cassabanana (*Sicana odorifera*) glandular trichomes. *Entomologia Experimentalis et Applicata*, **103**, 187–9.
155. Kessler, A., Halitschke, R., and Baldwin, I.T. (2004). Silencing the jasmonate cascade: induced plant defenses and insect populations. *Science*, **305**, 665–8.
156. Kester, K.M., Peterson, S.C., Hanson, F., Jackson, D.M., and Severson, R.F. (2002). The roles of nicotine and natural enemies in determining larval feeding site distributions of *Manduca sexta* L. and *Manduca quinquemaculata* (Haworth) on tobacco. *Chemoecology*, **12**, 1–10.
157. Kimmerer, T.W. and Potter, D.A. (1987). Nutritional quality of specific leaf tissues and selective feeding by a specialist leaf miner. *Oecologia*, **71**, 548–51.
158. Kirstine, W., Galbally, I., Ye, Y.R., and Hooper, M. (1998). Emissions of volatile organic compounds (primarily oxygenated species) from pasture. *Journal of Geophysical Research-Atmospheres*, **103**, 10605–19.
159. Klages. K., Donnison, H., Wünsche, J., and Boldingh, J. (2001). Diurnal changes in non-structural carbohydrates in leaves, phloem exudate and fruit in 'Braeburn' apple. *Australian Journal of Plant Physiology*, **28**, 131–9.
160. Kleiner, K.W., Raffa, K.F., and Dickson, R.E. (1999). Partitioning of ^{14}C-labeled photosynthate to allelochemicals and primary metabolites in source and sink leaves of aspen: evidence for secondary metabolite turnover. *Oecologia*, **119**, 408–18.
161. Klingauf, F. (1971). Die Wirkung des Glucosids Phlorizin auf das Wirtswahlverhalten von *Rhopalosiphum insertum* (Walk.) und *Aphis pomi* de Geer (Homoptera: Aphididae). *Zeitschrift für Angewandte Entomologie*, **68**, 41–55.
162. Kollner, T.G., Schnee, C., Gershenzon, J., and Degenhardt, J. (2004). The variability of sesquiterpenes cultivars emitted from two *Zea mays* cultivars is controlled by allelic variation of two terpene synthase genes encoding stereoselective multiple product enzymes. *Plant Cell*, **16**, 1115–31.
163. Koricheva, J. (1999). Interpreting phenotypic variation in plant allelochemistry: problems with the use of concentrations. *Oecologia*, **119**, 467–73.
164. Koricheva, J., Larsson, S., Haukioja, E., and Keinanen, M. (1998). Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. *Oikos*, **83**, 212–26.
165. Krischik, V.A. and Denno, R.F. (1983). Individual, populational, and geographic patterns in plant defense. In *Variable plants and herbivores in natural and managed systems* (ed. R.F. Denno and M.S. McClure), pp. 463–512. Academic Press, San Diego.
166. Krischik, V.A. and Denno, R.F. (1990). Patterns of growth, reproduction, defense, and herbivory inthe dioecious shrub *Baccharis halimifolia* (Compositae). *Oecologia*, **83**, 182–90.
167. Lance, D.R., Elkinton, J.S., and Schwalbe, C.P. (1986). Feeding rhythms of gypsy moth larvae;

effect of food quality during outbreaks. *Ecology*, **67**, 1650–4.

168. Lane, G.A., Biggs, D.R, Sutherland, O.W.R., Williams, E.M., Maindonald, J.M., and Donnell, D.J. (1985). Isoflavonoid feeding deterrents for *Costelytra zealandica*: structure–activity relationships. *Journal of Chemical Ecology*, **11**, 1713–35.
169. Langenheim, J.H. (1994). Higher plant terpenoids: a phytocentric overview of their ecological roles. *Journal of Chemical Ecology*, **20**, 1223–80.
170. Larsson, S. and Ohmart, C.P. (1988). Leaf age and larval performance of the leaf beetle, *Paropsis atomari. Evolutionary Entomology*, **13**, 19–24.
171. Lewinsohn, T.M. (1991). The geographical distribution of plant latex. *Chemoecology*, **2**, 64–8.
172. Lichtenthaler, H.K. (1999). The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**, 47–65.
173. Lombarkia, N. and Derridj, S. (2002). Incidence of apple fruit and leaf surface metabolites on *Cydia pomonella* oviposition. *Entomologia Experimentalis et Applicata*, **104**, 79–86.
174. Loughrin, J.H., Manukian, A., Heath, R.R., Turlings, T.C.J., and Tumlinson, J.H. (1994). Diurnal cycle of emission of induced volatile terpenoids herbivore-injured cotton plants. *Proceedings of the National Academy of Sciences of the USA*, **91**, 11836–40.
175. Lovett Doust, J. and Lovett Doust, L. (1985). Sex ratios, clonal growth and herbivory in *Rumex acetosella*. In *Studies on plant demography: a festschrift for John L. Harper* (ed. J. White), pp. 327–41. Academic Press, London.
176. Luckner, M. (1990). *Secondary metabolism in microorganisms, plants, and animals* (3rd edn). Springer, Berlin.
177. Deleted.
178. Macel, M., Vrieling, K., and Klinkhamer, P.G.L. (2004). Variation in pyrrolizidine alkaloid patterns of *Senecio jacobaea*. *Phytochemistry*, **65**, 865–73.
179. Malone, M., Palumbo, L., Boari, F., Monteleone, M., and Jones, H.G. (1994). The relationship between wound-induced proteinase inhibitors and hydraulic signals in tomato seedlings. *Plant Cell and Environment*, **17**, 81–7.
180. Marak, H.B., Biere, A., and van Damme, J.M.M. (2000). Direct and correlated responses to selection on iridoid glycosides in *Plantago lanceolata* L. *Journal of Evolutionary Biology*, **13**, 985–96.
181. Marion-Poll, F. and Descoins, C. (2002). Taste detection of phytoecdysteroids in larvae of *Bombyx mori, Spodoptera littoralis* and *Ostrinia nubilalis. Journal of Insect Physiology*, **48**, 467–76.
182. Martin, D., Tholl, D., Gershenzon, J., and Bohlmann, J. (2002). Methyl jasmonate induces traumatic resin ducts, terpenoid resin biosynthesis, and terpenoid accumulation in developing xylem of Norway spruce stems. *Plant Physiology*, **129**, 1003–18.
183. Matile, P. (1984). Das toxische Kompartiment der Pflanzenzell. *Naturwissenschaften*, **71**, 18–24.
184. Mattiacci, L., Dicke, M., and Posthumus, M.A. (1995). β-Glucosidase: an elicitor of herbivore-inducible plant odors that attract host-searching parasitic wasps. *Proceedings of the National Academy of Science of the USA*, **92**, 2036–46.
185. Mebs, D. and Schneider, M. (2002). Aristolochic acid content of South-East Asian troidine swallowtails (Lepidoptera: Papilionidae) and of *Aristolochia* plant species (Aristolochiaceae). *Chemoecology*, **12**, 11–13.
186. Mechaber, W.L., Marshall, DB., Mechaber, R.A., Jobe, R.T., and Chew, F.S. (1996). Mapping leaf surface landscapes. *Proceedings of the National Academy of Science of the USA*, **93**, 4600–3.
187. Meiners, T. and Hilker, M. (2000). Induction of plant synomones by oviposition of a phytophagous insect. *Journal of Chemical Ecology*, **26**, 221–32.
188. Merck Index (13th edn). (2001). Merck and Co., Rahway, NJ.
189. Mercke, P., Kappers, I.F., Verstappen, F.W.A., Vorst, O., Dicke, M., and Bouwmeester, H.J. (2004). Combined transcript and metabolite analysis reveals genes involved in spider mite induced volatile formation in cucumber plants. *Plant Physiology*, **135**, 2012–24.
190. Mitchell, B.K., Justus, K.A., and Asaoka, K. (1996). Deterrency and the variable caterpillar: *Trichoplusia ni* and sinigrin. *Entomologia Experimentalis et Applicata*, **80**, 27–31.
191. Mithen, R.F. (2001). Glucosinolates and their degradation products. *Advances in Botanical Research*, **35**, 214–62.
192. Mole, S. and Joern, A. (1993). Foliar phenolics of Nebraska sandhills prairie graminoids: between-years, seasonal, and interspecific variation. *Journal of Chemical Ecology*, **19**, 1861–74.
193. Mole, S., Ross, J.A.M., and Waterman, P.G. (1988). Light-induced variation in phenolic levels of foliage of rain-forest plants. I. Chemical changes. *Journal of Chemical Ecology*, **14**, 1–21.
194. Mordue (Luntz), A.J. and Blackwell, A. (1993). Azadirachtin: an update. *Journal of Insect Physiology*, **39**, 903–24.

195. Musser, R.O., Hum-Musser, S.M., Eichenseer, H., Peiffer, M., Ervin, G., Murphy, J.B., *et al.* (2002) Caterpillar saliva beats plant defences—a new weapon emerges in the evolutionary arms race between plants and herbivores. *Nature*, **416**, 599–600.

196. Muthukrishnan, J. and Selvan, S. (1993). Fertilization affects leaf consumption and utilization by *Porthesia scintillans* Walker (Lepidoptera: Lymantridae). *Annals of the Entomological Society of America*, **86**, 173–8.

197. Mutikainen, P., Walls, M., Ovaska, J., Keinänen, M., Julkunen-Tiitto, R., and Vapaavuori, E. (2002). Costs of herbivore resistance in clonal saplings of *Betula pendula*. *Oecologia*, **133**, 364–71.

198. Myers, J.H. (1985). Effect of physiological condition of the host plant on the ovipositional choice of the cabbage white butterfly *Pieris rapae*. *Journal of Animal Ecology*, **54**: 193–204.

199. Nault, L.R. and Styer, W.E. (1972). Effects of sinigrin on host selection by aphids. *Entomologia Experimentalis et Applicata*, **15**, 423–37.

200. Neuvonen, S. and Haukioja, E. (1991). The effects of inducible resistance in host foliage on birch-feeding herbivores. In *Phytochemical induction by herbivores* (ed. D.W. Tallamy and M.J. Raupp), pp. 277–91. John Wiley, New York.

201. Nitao, J.J., Zangerl, A.R., and Berenbaum, M.R. (2002). CNB: requiescat in pace? *Oikos*, **98**, 540–6.

202. Nykanen, H. and Koricheva, J. (2004). Damage-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. *Oikos*, **104**, 247–68.

203. O'Donnell, P.J., Calvert, C., Atzorn, R., Wasternack, C., Leyser, H.M.O., and Bowles, D.J. (1996). Ethylene as a signal mediating the wound response of tomato plants. *Science*, **274**, 1914–17.

204. Ogutuga, D.B.A. (1975). Chemical composition and potential commercial use of kola nut, *Cola nitida*, Vent. Schott and Endlicher. *Ghana Journal of Agricultural Science*, **8**, 121–5.

205. Okolie, P.N. and Obasi, B.N. (1993). Diurnal variation of cyanogenic glucosides, thiocyanate and rhodanese in cassava. *Phytochemistry*, **33**, 775–8.

206. Orians, C.M., Pomerleau, J., and Ricco, R. (2000). Vascular architecture generates fine scale variation in systemic induction of proteinase inhibitors in tomato. *Journal of Chemical Ecology*, **26**, 471–85.

207. Orozco-Cardenas, M., McGurl, B., and Ryan, C.A. (1993). Expression of an antisense prosystemin gene in tomato plants reduces resistance toward *Manduca sexta* larvae. *Proceedings of the National Academy of Sciences of the USA*, **90**, 8273–6.

208. Panzuto, M., Lorenzetti, F., Mauffette, Y., and Albert, P.J. (2001). Perception of aspen and sun/shade sugar maple leaf soluble extracts by larvae of *Malacosoma disstria*. *Journal of Chemical Ecology*, **27**, 1963–78.

209. Paul, M.J. and Cockburn, W. (1989). Pinitol, a compatible solute in *Mesembryanthemum crystallinum* L. *Journal of Experimental Botany*, **40**, 1093–8.

210. Pieterse, C.M.J. and Van Loon, L.C. (1999). Salicylic acid-independent plant defence pathways. *Trends in Plant Science*, **4**, 52–8.

211. Pourmohseni, H., Ibenthal, W.D., Machinek, R., Remberg, G., and Vray, V. (1993). Cyanoglucosidases in the epidermis of *Hordeum vulgare*. *Phytochemistry*, **33**, 295–7.

212. Preston, C.A., Laue, G., and Baldwin, I.T. (2001). Methyl jasmonate is blowing in the wind, but can it act as a plant–plant airborne signal? *Biochemical Systematics and Ecology*, **29**, 1007–23.

213. Rausher, M.D. (1992). Natural selection and the evolution of plant–insect interactions. In *Insect chemical ecology* (ed. B.D. Roitberg and M.B. Isman), pp. 20–88. Chapman & Hall, New York.

214. Rees, C.J.C. (1969). Chemoreceptor specificity associated with choice of feeding site by the beetle, *Chrysolina brunsvicensis* on its foodplant, *Hypericum hirsutum*. *Entomologia Experimentalis et Applicata*, **12**: 565–83.

215. Renwick, J.A.A. (2002). The chemical world of crucivores: lures, treats and traps. *Entomologia Experimentalis et Applicata*, **104**, 35–42.

216. Reymond, P., Weber, H., Damond, M., and Farmer, E.E. (2000). Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. *Plant Cell*, **12**, 707–19.

217. Reymond, P., Bodenhausen, N., Van Poecke, R.M.P., Krishnamurthy, V., Dicke, M., and Farmer, E.E. (2004). A conserved transcript pattern in response to a specialist and a generalist herbivore. *Plant Cell*, **16**, 3132–47.

218. Rhoades, D.F. (1977). The antiherbivore chemistry of *Larrea*. In *Creosote bush: biology and chemistry of* Larrea *in new world deserts* (ed. T.J. Mabry, J.H. Hunziker, and D.R. DiFeo), pp. 135–77. Dowden, Hutchinson & Ross, Stroutberg.

219. Rhoades, D.F. (1983). Responses of alder and willow to attack by tent caterpillars and webworms: evidence for pheromonal sensitivity of willows. In *Plant resistance to insects* (ed. P.A. Hedin), pp. 55–68. American Chemical Society Symposium 208, Washington, DC.

220. Robinson, T. (1974). Metabolism and function of alkaloids in plants. *Science*, **184**, 430–5.

221. Roda, A.L. and Baldwin, I.T. (2003). Molecular technology reveals how the induced direct defenses of plants work. *Basic and Applied Ecology*, **4**, 15–26.
222. Romani, A., Pinelli, P., Mulinacci, N., Vincieri, F.F., Gravano, E., and Tattini, M. (2000). HPLC analysis of flavonoids and secoiridoids in leaves of *Ligustrum vulgare* L. (Oleaceae). *Journal of Agricultural and Food Chemistry*, **48**, 4091–6.
223. Romeo, J.T., Ibrahim, R., Varin, L., and De Luca, V. (ed.). (2000). Evolution of metabolic pathways. *Recent Advances in Phytochemistry* **34**, 1–480.
224. Rosenthal, G.A. and Berenbaum, M.R. (ed.) (1991–1992). *Herbivores. Their interactions with secondary plant metabolites*, 2 Vols (2nd edn). Academic Press, San Diego.
225. Rosenthal, G.A. and Janzen, D.H. (ed.) (1979). *Herbivores. Their interaction with secondary plant metabolites*. Academic Press, San Diego.
226. Rowe, W.J. and Potter, D.A. (2000). Shading effects on susceptibility of *Rosa* spp. to defoliation by *Popillia japonica* (Coleoptera: Scarabaeidae). *Environmental Entomology*, **29**, 502–8.
227. Ruuhola, T.M. and Julkunen-Tiitto, M.R.K. (2000). Salicylates of intact *Salix myrsinifolia* plantlets do not undergo rapid metabolic turnover. *Plant Physiology*, **122**, 895–905.
228. Ryan, C.A. (2000). The systemin signaling pathway: differential activation of plant defensive genes. *Biochimica et Biophysica Acta—Protein Structure and Molecular Enzymology*, **1477**, 112–21.
229. Ryan, J.D., Gregory, P., and Tingey, T.M. (1982). Phenolic oxidase activities in glandular trichomes of *Solanum berthaultii. Phytochemistry*, **21**, 1885–7.
230. Sanderson, G.W. (1972). The chemistry of tea and tea manufacturing. *Recent Advances in Phytochemistry*, **5**, 247–316.
231. Sandström, J. (2000). Nutritional quality of phloem sap in relation to host plant-alternation in the bird cherry-oat aphid. *Chemoecology*, **10**, 17–24.
232. Schmeller, T. and Wink, M. (1998). Utilization of alkaloids in modern medicine. In *Alkaloids. Biochemistry, ecology, and medical applications* (ed. M.F. Roberts and M. Wink), pp. 435–59. Plenum, New York.
233. Schmelz, E.A., Grebenok, R.J., Ohnmeiss, T.E., and Bowers, W.S. (2000). Phytoecdysteroid turnover in spinach: long-term stability supports a plant defense hypothesis. *Journal of Chemical Ecology*, **26**, 2883–96.
234. Schultz, J.C. (1983). Habitat selection and foraging tactics of caterpillars in heterogeneous trees. In *Variable plants and herbivores in natural and managed systems* (ed. R.F. Denno and M.S. McClure), pp. 61–90. Academic Press, San Diego.
235. Schwab, W. (2003). Metabolome diversity: too few genes, too many metabolites? *Phytochemistry*, **62**, 837–49.
236. Scriber, J.M. (1984). Nitrogen nutrition of plants and insect invasion. In *Nitrogen in crop production* (ed. R.D. Hack), pp. 175–228. American Society of Agronomy, Madison, WI.
237. Sefton, M.A., Francis, I.L., and Williams, P.J. (1994). Free and bound volatile secondary metabolites of *Vitis vinifera* grape cv. Sauvignon blanc. *Journal of Food Science*, **59**, 142–7.
238. Seigler, D.S. and Conn, E.E. (1979). Cited by D.H. Janzen in Ref. 225.
239. Sharkey, T.D. and Yeh, S. (2001). Isoprene emission from plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **52**, 407–36.
240. Simmonds, M.S.J. (2001). Importance of flavonoids in insect–plant interactions: feeding and oviposition. *Phytochemsitry*, **56**, 245–52.
241. Simms, E.L. (1992). Costs of plant resistance to herbivory. In *Plant resistance to herbivores and pathogens* (ed. R.S. Fritz and E.L. Simms), pp. 392–425. University of Chicago Press, Chicago.
242. Sinden, S.L., Schalk, J.M., and Osman, S.F. (1978). Effects of daylength and maturity of tomato plants on tomatine content and resistance to the Colorado potato beetle. *Journal of the American Society of Horticultural Science*, **103**, 596–600.
243. Sirvent, T.M., Krasnoff, S.B., and Gibson, D.M. (2003). Induction of hypericins and hyperforins in *Hypericum perforatum* in response to damage by herbivores. *Journal of Chemical Ecology*, **29**, 2667–91.
244. Sisson, V.A. and Saunders, J.A. (1982). Alkaloid composition of the USDA tobacco (*Nicotiana tabacum* L.) introduction collection. *Tobacco Science*, **26**, 117–20.
245. Smiley, J.T., Horn, J.M., and Rank, N.E. (1985). Ecological effects of salicin at three trophic levels: new problems from old adaptations. *Science*, **229**, 649–51.
246. Smith, P.M. (1976). *The chemotaxonomy of plants*. Edward Arnold, London.
247. Soldaat, L.L., Boutin, J.P., and Derridj, S. (1996). Species-specific composition of free amino acids from the leaf surface of four *Senecio* species. *Journal of Chemical Ecology*, **22**, 1–12.
248. Southwell, I.A. and Bourke, C.A. (2001). Seasonal variation in hypericin content of *Hypericum perforatum* L. (St John's wort). *Phytochemistry*, **56**, 437–41.

249. Städler, E. and Roessingh, P. (1991). Perception of surface chemicals by feeding and ovipositing insects. *Symposia Biologica Hungarica*, **39**, 71–86.

250. Stanjek, V., Herhaus, C., Ritgen, U., Boland, W., and Städler, E. (1997). Changes in the leaf surface chemistry of *Apium graveolens* (Apiaceae) stimulated by jasmonic acid and perceived by a specialist insect. *Helvetica Chimica Acta*, **80**, 1408–20.

251. Storer, J.R., Elmore, J.S., and van Emden, H.F. (1993). Airborne volatiles from the foliage of three cultivars of autumn flowering chrysanthemums. *Phytochemistry*, **34**, 1489–92.

252. Stowe, K.A. (1998). Realized defense of artificially selected lines of *Brassica rapa*: effects of quantitative variation in foliar glucosinolate concentration. *Environmental Entomology*, **27**, 1166–74.

253. Strauss, S.Y., Rudgers, J.A., Lau, J.A., and Irwin, R.E. (2002). Direct and ecological costs of resistance to herbivory. *Trends in Ecology and Evolution*, **17**, 278–85.

254. Suomela, J., Kaitaniemi, P., and Nilson, A. (1995). Systematic within-tree variation in mountain birch leaf quality for a geometrid, *Epirrita autumnata. Ecological Entomology*, **20**, 283–92.

255. Svoboda, G.H. (1961). Alkaloids of *Vinca rosea* (*Catharanthus roseus*). IX. Extraction and characterization of leurosidine and leurocristine. *Lloydia*, **24**, 173–8.

256. Swain, T. (1979). Tannins and lignins. In *Herbivores. Their interaction with secondary plant metabolites* (ed. G.A. Rosenthal and D.H. Janzen), pp. 657–82. Academic Press, New York.

257. Tallamy, D.W., Stull, J., Ehresman, N.P., Gorski, P.M., and Mason, C.E. (1997). Cucurbitacins as feeding and oviposition deterrents to insects. *Environmental Entomology*, **26**, 678–83.

258. Thaler, J.S., Fidantsef, A.L., and Bostock, R.M. (2002). Antagonism between jasmonate- and salicylate-mediated induced plant resistance: effects of concentration and timing of elicitation on defense-related proteins, herbivore, and pathogen performance in tomato. *Journal of Chemical Ecology*, **28**, 1131–59.

259. Thieme, H. and Benecke, R. (1971). Die Phenylglykoside der Salicaceen. Untersuchungen über die Glykosidakkumulation in einigen mitteleuropäischen *Populus* -Arten. *Die Pharmazie*, **26**, 227–31.

260. Tscharntke, T., Thiessen, S., Dolch, R., and Boland, W. (2001). Herbivory, induced resistance, and interplant signal transfer in *Alnus glutinosa. Biochemical Systematics and Ecology*, **29**, 1025–47.

261. Turlings, T.C.J. (1994). The active role of plants in the foraging successes of entomophagous insects. *Norwegian Journal of Agricultural Sciences*, **16**, 211–19.

262. Turlings, T.C.J. and Tumlinson, J.H. (1992). Systemic release of chemical signals by herbivore-injured corn. *Proceedings of the National Academy of Sciences of the USA*, **89**, 8399–402.

263. Turlings, T.C.J., Tumlinson, J.H., and Lewis, W.J. (1990). Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, **250**, 1251–3.

264. Turlings, T.C.J., Loughrin, J.H., McCall, P.J., Rose, U.S.R., Lewis, W.J., and Tumlinson, J.H. (1995). How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences of the USA*, **92**, 4169–74.

265. Turlings, T.C.J., Alborn, H.T., Loughrin, J.H., and Tumlinson, J.H. (2000). Volicitin, an elicitor of maize volatiles in oral secretion of *Spodoptera exigua*: isolation and bioactivity. *Journal of Chemical Ecology*, **26**, 189–202.

266. Van Beek, T.A. and de Groot, AE. (1986). Terpenoid antifeedants, part I. An overview of terpenoid antifeedants of natural origin. *Recueil des Travaux Chimiques des Pays Bas*, **105**, 513–27.

267. Van Dam, N.M. and Vrieling, K. (1994). Genetic variation in constitutive and inducible pyrrolizidine alkaloid levels in *Cynoglossum officinale* L. *Oecologia*, **99**, 374–8.

268. Van Dam, N.M., Verpoorte, R., and van der Meijden, E. (1994). Extreme differences in pyrrolizidine alkaloid levels between leaves of *Cynoglossum officinale*. *Phytochemistry*, **27**, 1013–16.

269. Van Dam, N.M., Witte, L., Theuring, C., and Hartmann, T. (1995). Distribution, biosynthesis, and turnover of pyrrolizidine alkaloids in *Cynoglossum officinale* L. *Phytochemistry*, **39**, 287–92.

270. Van Dam, N.M., De Jong, T.J., Iwasa, Y., and Kubo, T. (1996). Optimal distribution of defenses: are plants smart investors? *Functional Ecology*, **10**, 128–36.

271. Van den Boom, C.E.M., Van Beek, T.A., Posthumus, M.A., De Groot, AE., and Dicke, M. (2004). Qualitative and quantitative variation among volatile profiles induced by *Tetranychus urticae* feeding on plants from various families. *Journal of Chemical Ecology*, **30**, 69–89.

272. Van Loon, J.J.A., Blaakmeer, A., Griepink, F.C., van Beek, T.A., Schoonhoven, L.M., and de Groot, AE. (1992). Leaf surface compound from *Brassica oleracea*

(Cruciferae) induces oviposition by *Pieris brassicae* (Lepidoptera: Pieridae). *Chemoecology*, **3**, 39–44.

273. Van Loon, J.J.A., Wang, C.-Z., Nielsen, J.K., Gols, R., and Qiu, Y.-T. (2002). Flavonoids from cabbage are feeding stimulants for diamondback moth larvae additional to glucosinolates: chemoreception and behaviour. *Entomologia Experimentalis et Applicata*, **104**, 27–34.
274. Van Oosten, V.R., De Vos, M., Van Pelt, J.A., Van Loon, L.C., Van Poecke, R.M.P., Dicke, M., *et al.* (2004). Signal signature of *Arabidopsis* induced upon pathogen and insect attack. In *Biology of plant–microbe interactions*, Vol. 4 (ed. I. Tikhonovich, B. Lugtenberg, and N. Provorov), pp. 199–202. International Society for Molecular Plant–Microbe Interactions, St Paul, MN.
275. Van Poecke, R.M.P. and Dicke, M. (2002). Induced parasitoid attraction by *Arabidopsis thaliana*: involvement of the octadecanoid and the salicylic acid pathway. *Journal of Experimental Botany*, **53**, 1793–9.
276. Van Poecke, R.M.P., Posthumus, M.A., and Dicke, M. (2001). Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: chemical, behavioral, and gene-expression analysis. *Journal of Chemical Ecology*, **27**, 1911–28.
277. Vandenberg, P. and Matzinger, D.F. (1970). Genetic diversity and heterosis in *Nicotiana*. III. Crosses among tobacco introductions and flue-cured varieties. *Crop Science*, **10**, 437–40.
278. Verschaffelt, E. (1910). The cause determining the selection of food in some herbivorous insects. *Proceedings Koninklijke Nederlandse Akademie van Wetenschappen*, **13**, 536–42. (Reprinted in *Proceedings Koninklijke Nederlandse Akademie van Wetenschappen*, **100**, 362–68, 1997)
279. Vickery, M.L. and Vickery, B. (1981). *Secondary plant metabolism*. Macmillan, London.
280. Visser, J.H. and Avé, D.A. (1978). General green leaf volatiles in the olfactory orientation of the Colorado potato beetle, *Leptinotarsa decemlineata*. *Entomologia Experimentalis et Applicata*, **24**, 738–49.
281. Vrieling, K. and Van Wijk, A.M. (1994). Cost assessment of the production of pyrrolizidine alkaloids in ragwort (*Senecio jacobaea* L.). *Oecologia*, **97**, 541–6.
282. Vuorinen, T., Reddy, G.V.P., Nerg, A.M., and Holopainen, J.K. (2004). Monoterpene and herbivore-induced emissions from cabbage plants grown at elevated atmospheric CO_2 concentration. *Atmospheric Environment*, **38**, 675–82.
283. Walker, K. , Long, R., and Croteau, R. (2002). The final acylation step in taxol biosynthesis: cloning of the taxoid C13-side-chain *N*-benzoyltransferase from *Taxus*. *Proceedings of the National Academy of Science of the USA*, **99**, 9166–71.
284. Waterman, P.G. and Mole, S. (1989). Extrinsic factors influencing production of secondary metabolites in plants. In *Insect–plant interactions*, Vol. 5 (ed. E.A. Bernays), pp. 107–34. CRC Press, Boca Raton.
285. Waterman, P.G. and Mole, S. (1994). *Analysis of phenolic plant metabolites*. Blackwell, Oxford.
286. Watson, M.A. (1986). Integrated physiological units in plants. *Trends in Ecology and Evolution*, **1**, 119–23.
287. Webber, B.L. and Woodrow, I.E. (2004). Cassowary frugivory, seed defleshing and fruit fly infestation influence the transition from seed to seedling in the rare Australian rainforest tree, *Ryparosa* sp. nov. 1 (Achariaceae). *Functional Plant Biology*, **31**, 505–16.
288. White, T.C.R. (1970). The nymphal stage of *Cardiaspina densitexta* (Homoptera: Psyllidae) on leaves of *Eucalyptus fasciculosa*. *Australian Journal of Zoology*, **18**, 273–3.
289. Whitham, T.G. (1983). Host manipulation of parasites: within-plant variation as a defense against rapidly evolving pests. In *Variable plants and herbivores in natural and managed systems* (ed. R.F. Denno and M.S. McClure), pp. 15–41. Academic Press, San Diego.
290. Whittaker, R.H. (1970). The biochemical ecology of higher plants. In *Chemical ecology* (ed. E. Sondheimer and J.B. Simeone), pp 43–70. Academic Press, New York.
291. Wittstock, U. and Halkier, B.A. (2002). Glucosinolate research in the *Arabidopsis* era. *Trends in Plant Science*, **7**, 263–70.
292. Wildon, D.C., Thain, J.F., Minchin, P.E.H., Gubb, I.R., Reilly, A.J., Skipper, Y.D., *et al.* (1992). Electrical signalling and systemic proteinase inhibitor induction in the wounded plant. *Nature*, **360**, 62–5.
293. Wink. M. (1998). Chemical ecology of alkaloids. In *Alkaloids. Biochemistry, ecology and medicinal applications* (ed. M.F. Roberts and M. Wink), pp. 265–326. Plenum, New York.
294. Wink, M. and Roberts, M.F. (1998). Compartmentation of alkaloid synthesis, transport, and storage. In *Alkaloids. Biochemistry, ecology and medicinal applications* (ed. M.F. Roberts and M. Wink), pp. 239–62. Plenum, New York.

295. Wool, D. and Bogen, R. (1999). Ecology of the gall-forming aphid, *Slavum wertheimae*, on *Pistacia atlantica*: population dynamics and differential herbivory. *Israel Journal of Zoology*, **45**, 247–60.
296. Woodhead, S. and Chapman, R.F. (1986). Insect behaviour and the chemistry of plant surface waxes. In *Insects and the plant surface* (ed. B.E. Juniper and T.R.E. Southwood), pp. 123–35. Edward Arnold, London.
297. Zangerl, A.R. and Bazzaz, F.A. (1992). Theory and pattern in plant defense allocation. In *Plant resistance to herbivores and pathogens* (ed. R.S. Fritz and E.L. Simms), pp. 363–91. University of Chicago Press, Chicago.
298. Zucker, W.V. (1982). How aphids choose leaves: the role of phenolics in host selection by a galling aphid. *Ecology*, **63**, 972–81.

CHAPTER 5

Plants as insect food: not the ideal

The subject of this chapter—plants as food for herbivorous insects—touches the heart of insect–plant biology. Its theme can be captured in two basic questions. First, what do plants offer to insects by way of nutrition? And second, what do insects need for optimal growth and reproduction? Answering these two questions is seriously hindered by the fact that (1) the chemical composition of plants, as noted before, varies among species in both space and time, and (2) the nutritional requirements of insects vary between species and with developmental stage and environmental conditions. Apart from these complications, a major nutritional discrepancy clearly exists between what plants provide and what insects require. Plants appear to supply food that is at best of marginal quality. What is the basis of this statement and how do we know?

As insects, like all animals, need food as material for conversion into body substance and as a source of energy, it is appropriate to compare the chemical composition of insects with that of plants. Figure 5.1 shows the concentrations of some major elements in insects and plants.

For four of the seven macro-elements and three of the four micro-elements, average concentrations found in insects are substantially higher than those found in plants. Nitrogen deserves special attention because, relative to the other major components of living organisms, a substantial part of plant nitrogen occurs in inorganic form, which is not utilizable to insects, although exceptions may occur.[85] Whereas the nitrogen content of animals amounts to about 8–14% of their (dry) bodyweight, plants usually contain only 2–4% total nitrogen (Fig. 5.2).

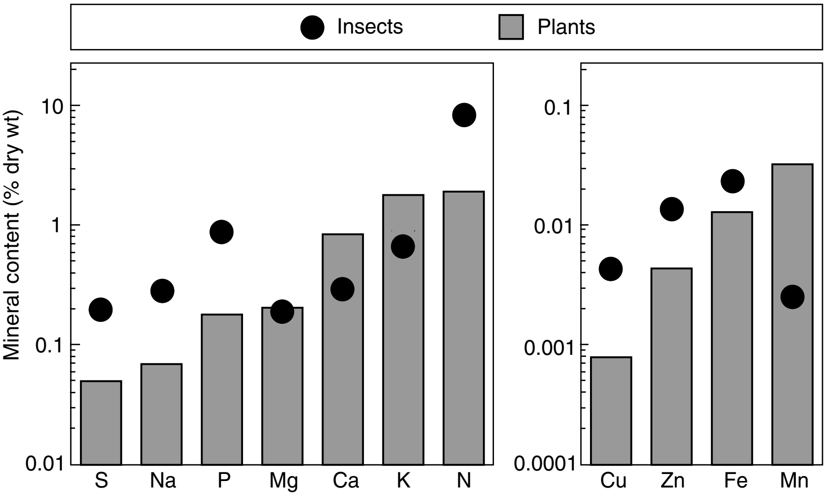


Figure 5.1 Average concentrations of elements in plant tissues compared with those in insects. It should be emphasized that the levels presented for plants, in particular, vary greatly between species. Environmental factors and plant (tissue) age cause further interspecific and intraspecific variation; see Figure 5.2 for nitrogen. The vertical scale is logarithmic. (Data from Allen *et al.*, 1974.)[4]

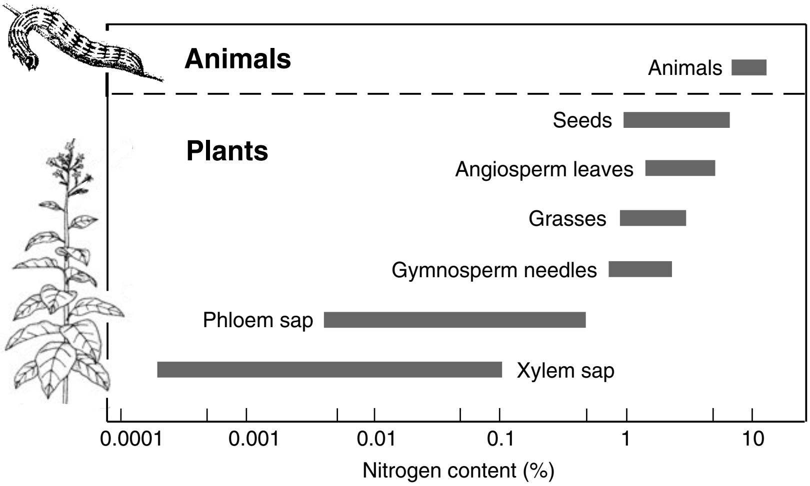


Figure 5.2 Variations in nitrogen concentration (% dry weight) of different plant parts compared with that in animals. Xylem and phloem sap concentrations are expressed as nitrogen weight/volume. (From Mattson, 1980.)[112]

Likewise, the caloric value of animal tissue (in insects 22.8 J/mg) exceeds that of plants (terrestrial plants on average 18.9 J/mg). These ratios show that herbivorous insects must concentrate nitrogen when converting plant food into body tissue. Attaining the caloric value typical of insects requires less grading up. Therefore, the nutritional value of a plant for an insect (more so than for mammals, which grow much more slowly, but use more energy) is determined primarily by its nitrogen content, whereas its caloric value is of less importance (Table 5.1).

Table 5.1 Approximate optimal ratios of protein to available carbohydrate plus fat (expressed as grams of glucose) in the diets of some herbivorous insects compared with some mammals (modified after Bernays, 1982)[25]

Animal	Ratio of protein to glucose
Silkworm	1 : 3
Silkworm (artificial diet)	1 : 1.5
Locust (artificial diet)	1 : 1
Cabbage butterfly larva	1 : 1
Calf (very young)	1 : 4
Cow	1 : 7
Buffalo	1 : 10
Goat	1 : 15

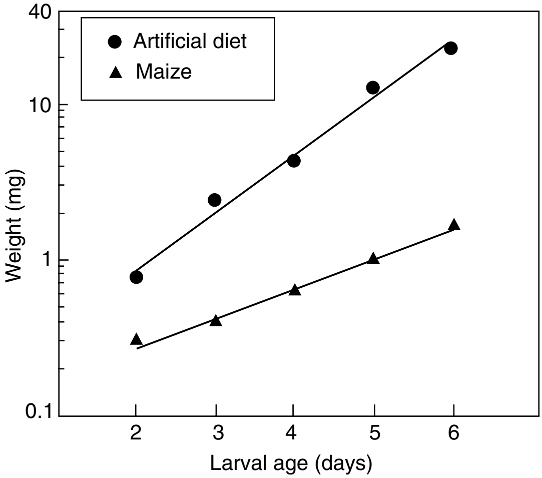


Figure 5.3 Larval growth of *Agrotis ipsilon* over 6 days after hatching on susceptible maize plants or on artificial diet. Note that the *y*-axis (larval bodyweight) is a logarithmic scale. (From Reese and Field, 1986.)[136]

5.1 Plants are suboptimal food

The food of insect herbivores consists of dilute nutrients in a matrix of indigestible structural compounds, such as cellulose and lignin, and a variety of allelochemicals (which in many cases exert toxic effects, interfere negatively with digestion, or deter feeding). To make things worse, the quantitative ratios of nutrients present in the plant may differ greatly from those required by the insect. Qualitatively the nutritional requirements of insects are generally the same as for other animals, except that, unlike many other animals, insects lack the capacity to synthesize sterols. Therefore, they must extract sterols together with several other essential nutrients (amino acids, carbohydrates, lipids, fatty acids, vitamins, trace elements) from their food.[19] The nutritional requirements of different insect species are often fairly specific and may allow for only small margins, qualitatively as well as quantitatively. Optimal growth, survival, and fecundity require certain protein : carbohydrate ratios, which may vary considerably among species and developmental stages. Polyphagous larvae of the corn earworm (*Helicoverpa zea*) grow best on an artificial diet with a protein : carbohydrate ratio of 79 : 21. Conversely, *Locusta migratoria* nymphs require a totally different ratio of 50 : 50.[155] This striking contrast results from differences in organism characteristics.[177] Whereas corn earworms grow fast and therefore need protein-rich food, the locust is characterized by slower growth and higher activity levels, hence the need for a high intake of energy to supply its muscles with fuel.

When plants are truly suboptimal food, owing to inadequate nutrient ratios and the presence of allelochemicals that need to be detoxified, it should be possible to develop artificial diets that support growth better than natural food plants. Young cutworm larvae (*Agrotis ipsilon*) raised on an artificial diet did indeed gain 12 times as much weight as those raised on tissues from 'susceptible' corn leaves, their natural food (Fig. 5.3).[136]

This observation and similar results from other insect species, which grew faster, attained higher pupal weights, and showed better reproduction on artificial diets, prove that the most susceptible plants are in fact remarkably well defended against insect attack[136] and are poor food sources from a nutritional point of view. Given this conclusion Berenbaum[22] has presented an intriguing hypothesis based on the contention that nutritional inadequacy may be a major determinant of host-plant resistance. In that case the selective impact of herbivory may have been a driving factor in establishing a biosynthetic and structural diversity of primary metabolites that would render plants less suitable food sources for herbivores. Unfortunately, Berenbaum's hypothesis cannot be proved at present. On the contrary, there is growing evidence that the main characteristics of plant metabolism evolved before the appearance of terrestrial plants. However, in view of the great variety of effects that insects have had on the plant world over the ages, it could well be that insects have significantly affected the evolution of ancient plant traits such as the basic biochemical processes of metabolism.

5.1.1 Nitrogen

The importance of organic nitrogen for normal insect growth and reproductive success cannot be overemphasized. In spite of its general occurrence in the atmosphere, nitrogen is, of all the elements essential to organic life on Earth, the one that is least available in a usable form, that is, combined with other chemicals. Proteins are the basic structural materials of insects, not only of soft tissues but also of the integument. Cuticular proteins usually make up more than 50% of cuticle by dry weight. In contrast, the bulk of plant tissue consists of carbohydrates, as major components of cell walls include cellulose and hemicellulose, in addition to lignin, cutin, silica, and cell wall protein. Moreover, the balance of amino acids that constitute plant proteins differs from the dietary requirements of insects (Fig. 5.4). As large amounts of aromatic compounds bind the cuticular proteins together, insects need considerably higher levels of aromatic amino acids, such as phenylalanine and tryptophan, than are present in plant proteins.[25,40]

The amount of nitrogen in plants (a correlate of protein content) varies enormously with species, organ, season, and other environmental factors. Generally the leaves of forbs are richer in soluble proteins than deciduous species and graminoids, whereas evergreens have the lowest amounts. An analysis of 72 literature reports showed large differences in midsummer nitrogen levels in the leaves of plants belonging to eight different families. Woody species in the Ericaceae and Myrtaceae contained low levels of protein, whereas the highest levels were recorded in herbaceous legumes.[28] Such differences undoubtedly determine the (un)suitability of a particular plant for certain insect herbivores. Thus, two polyphagous thrips species were found in a botanical collection grown in a glasshouse to infest plant species with moderate to high leaf nitrogen levels, whereas low-nitrogen species appeared unsuitable for growth and development.[145]

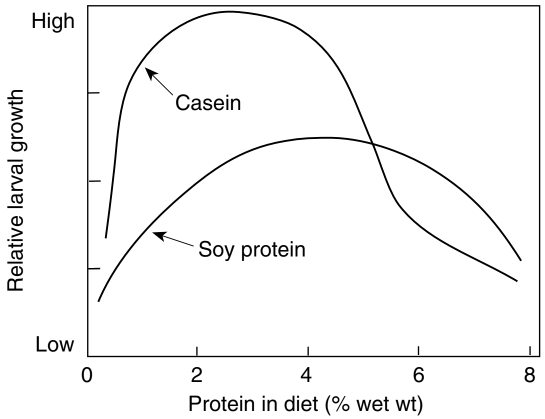


Figure 5.4 Ability of two dietary proteins to support larval growth of *Spodoptera exigua*, a polyphagous noctuid species. Growth with the various protein regimens is shown relative to the growth of controls on a standard artificial diet containing 2.4% wet weight of casein. Higher soy protein levels are needed to obtain maximal growth than on casein diets. The amino acid composition of the plant protein (soybean) is less adequate for insect growth than that of the animal-derived protein. (From Duffey *et al.*, 1986.)[59]

The growth efficiency of a variety of insects is closely related to plant nitrogen content, a correlate of protein content. As the nitrogen content of their food increases, insects become more efficient in converting plant material into body tissue (Fig. 5.5).

Thus insects on plants with 1% nitrogen require over three times more food than insects on plants containing 6% nitrogen.[112] Yet, despite its essential role in herbivore–plant relations, the total nitrogen content of a plant is frequently a poor index of its nutritional value. High nitrogen levels may coincide with metabolically useless nitrogen compounds, such as alkaloids, or with tannins, which in some cases reduce digestive efficiency (see Section 5.3.4). In this respect phloem-feeding species are in a more privileged position than chewing insects, because nearly all nitrogen-containing compounds in phloem sap can be utilized.[56]

Xylem forms a less suitable food source than phloem, as the nitrogen concentrations of xylem sap (less than 0.1%) are typically ten times lower

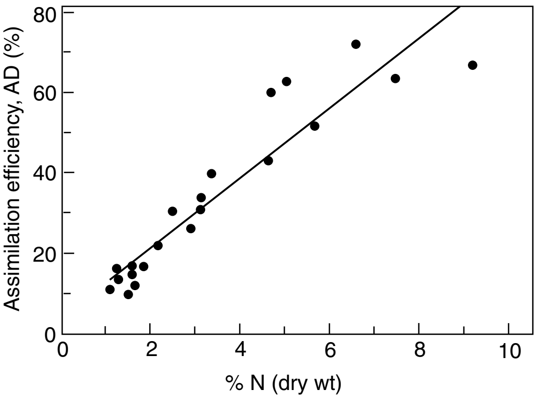


Figure 5.5 Assimilation efficiency of total dry matter as a function of total nitrogen levels in food plants of the plant bug *Leptoterna dolabrata*. (Redrawn from McNeill and Southwood, 1978.)[111]

than phloem concentrations and two orders of magnitude less than that of foliar tissues (see Fig. 5.2); leaf-hoppers (Cicadellidae) feeding on xylem must suck enormous amounts of sap to meet their nitrogen and carbohydrate demands. Feeding rates can be as high as 300 to 1000 times the bodyweight per day.[41] Despite this high flow rate through the alimentary canal, the efficiency of nutrient extraction reaches the phenomenal level of 99%. Some detailed measurements on leaf-hopper consumption of plant compounds showed that an individual insect consumes 3.9 ml water, 57 μmol organic carbon, and 21 μmol organic nitrogen during a day of feeding. The daily intake of carbon corresponds with about 14% of the amount of carbon present in the body of this insect. For nitrogen, the daily intake is as high as 29% of its body nitrogen. From a plant perspective, the drain of water and nutrients may be problematic. For instance, the amount of nitrogen extracted by 16 leaf-hoppers during a 3-week experiment amounted to 48% of total plant nitrogen in the case of a soybean (*Glycine max*) plant.[7]

Although many studies report on positive correlations between insect performance and host-plant nitrogen concentrations, a word of caution must be made to generalize results obtained from the larval phase only. In a more extensive study on larval growth and adult performance in a lepidopteran species, faster larval growth was found to go together with increased pupal mortality and a reduction of adult size. Insect performance as a whole is in this case not correlated with a higher growth rate during the larval stage on nitrogen-enriched host plants.[66]

Where nitrogen is truly an important indicator of food quality, or even the limiting factor[180] for growth, the application of nitrogen fertilizer to plants can be expected positively to affect herbivore performance. Such effects do indeed often occur (see Fig. 4.21), but negative effects have also been reported. Scriber,[147] on the basis of a literature survey, listed at least 115 studies in which insects grew better with increased plant nitrogen. On the other hand, at least 44 studies indicated a decrease in herbivore performance with high nitrogen concentration. These often contradictory responses of insects to changes in nitrogen content have been explained by several mechanisms. Probably, insects are physiologically adapted to nitrogen levels that are normal (or slightly higher than normal) for their normal host(s). When the nitrogen content of fir trees was manipulated by growing seedlings on nutrient solutions that differed in nitrogen concentration, the nitrogen content of the needles ranged from 0.7% to 5% (dry weight). Western spruce budworms (*Choristoneura occidentalis*) performed best at levels of around 2.5%, concentrations that are normally encountered in nature.[39] Optimum curves were also obtained with two noctuid species on artificial diets with varying amounts of protein (see Fig. 5.4).[59]

It has been conjectured that 'flush feeders', insect species that are adapted to high nitrogen levels in their food, would respond positively to an increased amount of nitrogen being transported to the growing tissues, whereas 'senescence feeders' would respond negatively to the decreased export of nitrogen from senescing tissues.[180] Moreover, nitrogen fertilization may cause many kinds of physiological and morphological alteration to plants and affect, amongst other things, secondary metabolism, resulting in increased production of defence substances. Susceptibility to plant pathogens and environmental factors, such as micro-climate and weed growth, may also alter. Morphological changes may include an increase in leaf surface and leaf thickness, changes in the length of internodes and toughness of veins, all of which could negatively affect herbivorous insects. Thus, nitrogen fertilization-induced changes naturally alter the value of the plant as a home for the herbivore and its natural enemies.[67]

The role of nitrogen, although of prime importance, cannot be appreciated separately from a plant's total chemical composition. This notion is evident from the finding that the effects of secondary plant substances, such as tannic acid and phenolics, on insect growth may strongly depend on carbohydrate to protein ratios in the diet.[78,155]

5.1.2 Water

Water is the cradle of life. Insects, like other animals, need it and acquiring sufficient amounts of water is a major nutritional 'hurdle' for most herbivorous species.[147,151] Even though water is not a nutrient according to traditional terminology, and the water content of foliage varies from 45% to 95% of fresh weight, the amount of water in the food of many lepidopterous larvae provides a surprisingly useful index of its nutritional value and thus of growth performance. The importance of sufficient leaf water content for an insect has been shown by an experiment in which larval growth rates were determined on various legumes and alfalfa cultivars differing in water content. Significantly better growth occurred on plants with higher water content. The relevance of dietary water was confirmed in experiments with artificial diets varying in water content.[147] When the normal amount of water in foliage drops, its nutritional value decreases. When caterpillars belonging to 16 species were fed on excised leaves without water supplementation via the petioles, their relative growth rates showed reductions of up to 40% even when the food did not show any indication of desiccation. Such effects were more pronounced for tree leaf-feeders than for forb leaf-feeders, which is probably due to the fact that the former group already has lower conversion efficiencies because of the naturally lower water and nitrogen contents of tree leaves

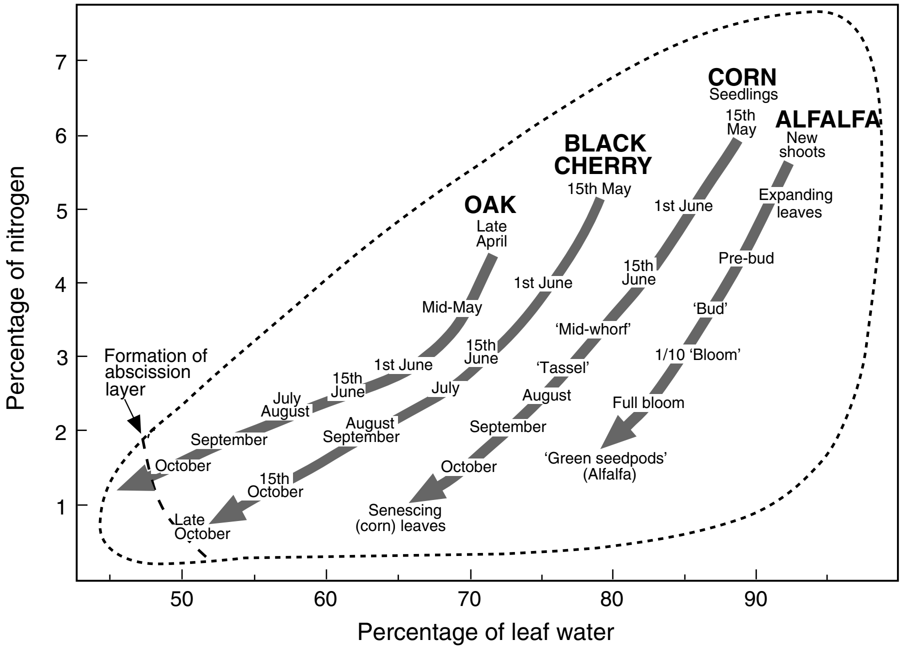


Figure 5.6 Nitrogen and water content of foliage of herbaceous plants and deciduous trees decreases as the season progresses, and consequently the nutritional value of plants is reduced compared with that of spring foliage. (From Scriber and Slansky, 1981;[148] reproduced, with permission, from the *Annual Review of Entomology*, Vol. 26, © 1981, by Annual Reviews, Inc.)

compared with herbal foliage.[82,146] Acridids, on the other hand, more easily tolerate reduced water content in their host plants. Water stress even made 12 of 41 plant species more palatable to desert locusts, whereas only five of 41 species became less palatable.[29]

Water content and leaf nitrogen levels (especially protein and amino acids) often co-vary, both being higher in young leaves than in mature and senescing leaves. The nutritional value of herbs and grasses with water contents of 80–90% and nitrogen levels of 5–6% is higher than that of foliage from woody plants, which typically contains 60% water and 2% nitrogen (see Section 5.4.2).[161] Superimposed on the differences between plant groups are seasonal changes in water (and nitrogen) content, with diminishing nutritional values as the summer progresses (Fig. 5.6).

Although herbivorous insects feed on material that contains a high proportion of water, many species can be seen to drink from dew drops or other sources of free water. Caterpillars can perceive water from a distance of at least up to 2 cm and will readily drink when encountering water drops.[51,116] In contrast to caterpillars, which easily evaporate water,[187] grasshoppers normally need an average water intake of only about 60% of their food. The importance of water to acridids is also evident from the finding that their state of hydration influences food selection. After being fed for some time on drier food sources, locusts eat more than normally from plants with a high water content to compensate for a water deficit.[138] Clearly, herbivores possess a behavioural regulation of water balance by selective feeding.

5.2 Artificial diets

When studying behavioural responses to specific plant compounds or the nutritional role of certain plant components, artificial diets of known chemical composition have been shown to be an indispensable tool. Plant material is difficult to standardize, because individual plants and plant parts may vary greatly, with season, developmental stage, etc. Artificial diet formulation allows for precise control of nutritional factors. Beginning in the 1950s, diets have been developed for many species. This proved to be more difficult than might be expected once the chemical composition of a plant is known and the nutritional requirements of its herbivores have been listed. The difficulties arise from two plant traits that are difficult to copy in an artificial diet. First, in spite of their high water content, which may amount to 90% of total weight, plants provide a dry substrate to their herbivores. Plant food is essentially a liquid packed in microcapsules (cells), giving it a dry outside. Second, the physical and chemical structure of these microcapsules prevents microorganisms from invading the highly nutritive cell contents. To mimic the firm surface of plant parts, artificial diets are given some rigidity by incorporating agar, cellulose, or other nutritionally inert substances that add texture to the liquid food. This also provides roughage, which aids the passage of the food material through the gut. To suppress bacterial and fungal decay, the food has to be sterilized by heat and by adding antibiotics. These compounds, however, may also affect the feeder through their impact on gut microbes and detoxification enzymes, or in other ways, and are often not tolerated. Therefore, finding an effective dose that is at the same time harmless to the insect consumer forms an important element in diet development. Because different species often differ slightly in their precise nutritional requirements, even small changes in diet composition can have drastic effects on insect growth and reproduction. Moreover, origin and storage conditions of the ingredients and variations in diet preparation can seriously affect its quality, even when polyphagous and less finicky insects are involved, as has been described in some detail for gypsy moth larvae (*Lymantria dispar*).[96]

Currently several excellent books on artificial diets exist that have a 'cookbook' style, giving step-by-step instructions to prepare diets for specific insect species.[49,159] Moreover, 'ready-to-eat' diets for several species are obtainable from commercial sources. Some artificial diets, lacking any host plant-specific chemical are suitable for a number of different insect species. They typically contain a protein source (casein and/or wheatgerm), sugars, lipids, sterols, minerals, and vitamins. Agar and cellulose function as gelling and bulking agents, and microbial inhibitors are added to inhibit growth of microorganisms. In the case of food specialists, addition of host-plant material is part of many successful diets, owing to the presence of either specific feeding stimuli or nutritional factors that are still unknown.[68]

Of course, artificial diets differ in many respects from natural food sources. The question arises whether insects reared on diets for many generations, or even for only one generation, exhibit changes in behaviour or physiology. When the European corn borer (*Ostrinia nubilalis*) was cultured continuously (for up to 153 generations) on an artificial diet, it showed a diminished ability to damage susceptible maize plants. However, when each time after seven generations on artificial diet one generation was raised on corn, the culture maintained its virulence for corn.[72] In another insect (corn earworm, *Helicoverpa zea*), however, vigour and food utilization on a host plant remained unchanged after more than 275 generations on an artificial diet.[48] Short-term changes may also occur. Insects grown on a relatively soft artificial diet may, due to reduced physical exercise compared with those feeding on tough plant leaves, develop differences in head musculature and dimensions. Such variations have been noted among caterpillars fed host plants differing in toughness.[26] Larvae of tobacco hornworms (*Manduca sexta*) raised on a diet are sluggish and less dextrous in holding themselves on vertical plant structures than conspecifics fed plant tissues. Therefore, quality control is an essential part of all insect-rearing procedures.[8,98]

5.3 Consumption and utilization

5.3.1 Food quantities eaten

Fast-growing insects consume large amounts of food. Their gut is shaped to process large food volumes and in non-reproductive stages occupies most of the body cavity. Food passage through the gut is fast and often takes only a few hours in leaf-feeding insects.[167] The locust *Schistocerca gregaria*, for instance, maintains a throughput time of 1.5 h while constantly feeding, but this drops rapidly during periods without food. Food transit time in aphids can be as short as 1 h, and is only slightly longer in a xylem-feeding cicadellid.[53] Young caterpillars may consume plant tissues at a rate of up to six times their bodyweight per day, whereas adult locusts eat daily about their own weight of food. Sap-feeding spittlebugs (Cercopidae) may even ingest xylem sap in amounts ranging from 100 to 1000 times their bodyweight per day.[164]

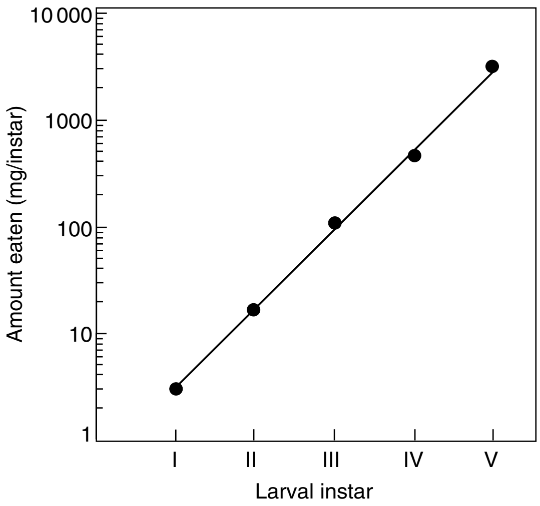


Figure 5.7 Food intake by silkworm (*Bombyx mori*) larvae over different instars. Note logarithmic scale of ordinate. (From Anantha Raman *et al.*, 1994.)[6]

The stepwise growth of insects makes it convenient to determine food intake and digestive efficiency during larval growth.[164] Growth follows an exponential increase in weight, with often more than a doubling of weight in each instar. The steep growth curve is parallelled by an exponential increase in food intake (Fig. 5.7).

As a result, mature larvae often weigh several thousands times their weight at hatching. Full-grown silkworm (*Bombyx mori*) larvae, for instance, weigh 10 000 times the bodyweight of neonates. Lepidopterous larvae consume 94–98% of all food during the penultimate and last stadia alone.[6]

5.3.2 Utilization

(a) Utilization as a factor driving host-plant use

A prime question in insect–plant studies is with what efficiency herbivores utilize their host plants nutritionally. To answer this, one needs to know which fraction of the total amount of food that is ingested is actually used for growth and ultimately for reproduction. A higher efficiency of food utilization can be seen as an indicator of higher nutritional quality of a plant or plant part. In conjunction with sensory and behavioural factors that determine host-plant specificity, differences in host-plant utilization efficiency may contribute to explain host-plant specificity. A considerable literature has accumulated in this field, often referred to as 'nutritional ecology'.[163] A landmark publication in the field of insect nutrition was the influential review article in 1968 by Waldbauer,[176] who summarized the earlier literature and proposed to standardize rigorously the quantitative methods and parameters employed in such approaches. The extensive literature that has appeared since 1968 has been summarized in several reviews and books.[161,163,164] The number of publications in the field of insect nutritional ecology that appeared in the period 1992–2003 showed a 3-fold increase compared with the number published from 1982 to 1991.[174] General conclusions from quantitative nutritional studies are that major herbivore guilds, such as tree and herb feeders, differ in their utilization efficiency (see below) and that, as stated above, water and nitrogen are primary determinants of nutritional quality irrespective of the group studied. However, when moving from the guild to the species level, assessing whether an insect species uses one plant species more efficiently than an alternative host plant, or whether a secondary plant substance affects utilization efficiency, accurate measurements are often more difficult to obtain than might be expected (see below).[174]

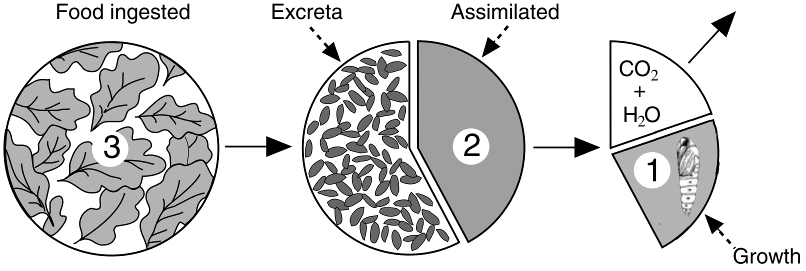
(b) Parameters of utilization and performance, and their interrelationships

Waldbauer[176] defined three parameters of utilization, now commonly termed nutritional indices:

1. *Approximate digestibility* (abbreviated as AD, also termed absorption efficiency)
2. *Efficiency of conversion of ingested food* to body substance (ECI, also termed growth efficiency)
3. *Efficiency of conversion of digested food* to body substance or utilization efficiency (ECD, also termed metabolic efficiency).

The prevalent method used to quantify food intake and utilization has been the *gravimetric method*, which involves weighing of food, body, and faecal masses at the start and end of the experimental period. It is based on the so-called *budget equation*, often given[130,142] as: $C = G + R + FU$, where C is the amount of food consumed, G is insect biomass produced (i.e. somatic and reproductive growth, and several secreted and excreted products that are not actually contributing to somatic growth *per se*, such as exuviae, silk, and digestive enzymes), R is respiration (amount of carbon dioxide respired), and FU is faeces (urinary wastes and other metabolic waste products egested, together with undigested food as faecal constituents). As an alternative, bicoordinate utilization plots[134] have met increased application over recent years.[74,155] These plots represent graphically the relationship between intake and various components of the nutrient budget given above, for either total intake or specific nutrient categories. Utilization plots avoid several problems that are associated with the use of the ratio-based nutritional indices.

Budget items are routinely expressed in dry matter units, because water escapes from food, faeces, and the insect body as vapour and the losses via these routes are technically difficult to quantify. The amount of dry matter lost by respiration is quantified directly in only few cases.[173] As a consequence, the accuracy of the budget cannot be checked and this has been a matter of debate, especially in the wider field of ecological energetics.[184] An important source of error is an inaccurate determination of the dry matter content of the food, either plant food or artificial diet.[10,141,172] As plant tissues respire during the experiment and because plant species and tissues differ in their respiration rates, losses due to plant respiration should be taken into account for reliable measurements of nutritional indices. Errors become more serious when an excess of food is offered, although some degree of excess is required to ensure an *ad libitum* situation.[168] For instance, when only half of the food presented to the feeding insect is consumed during the experiment and the percentage dry matter of, for example, leaf material is estimated only slightly incorrectly (e.g. at 14.5%, whereas it is actually 14%), the value of ECD is calculated to be 40% when in reality it is 50%. Because of the spatial heterogeneity of the dry matter content of leaves and other plant organs, these errors are of a random rather than a systematic nature and hence will hamper the experimenter from measuring differences reliably. When, in addition, leaf respiration rates are not accounted for appropriately, these errors may become even more serious and may lead to physiologically improbable degrees of variation in metabolic efficiency and consequently to erroneous conclusions (see also Section 5.3.2e).[172,174] Unfortunately, plant tissue respiration rates and proper controls have been taken into account in only very few cases.[173] Details of techniques, methods of measurement, and error analysis are beyond the scope of this text and can be found elsewhere.[98,172] The way in which AD, ECI, and ECD values are calculated from the gravimetric measurement of C, G and FU is depicted in Figure 5.8. Alternative methods to quantify food utilization are based on markers, elemental budgets, radiotracers, and gas analysis.[98,166,172] These techniques require sophisticated chemical or physical analytical equipment and have thus far been used in a small number of studies. The use of the doubly labelled water method[91] offers the possibility to study carbon dioxide production by organisms under natural foraging conditions, in which the condition of plants as a food source can be studied under realistic ecophysiological conditions. This technique has been validated and applied successfully to freely foraging bumble bees.[186] Realistic measurements of the nutritional quality of living plant tissue to an insect herbivore should be performed under natural light intensity, allowing for photosynthesis to occur. This condition has not been met in any of the gravimetric studies published to date.[174] Insect herbivory has been shown to affect photosynthetic rate in the majority of cases, by either increasing or decreasing the rate, depending on the feeding mode of the herbivore.[179] These effects cannot in all cases be mimicked by mechanical damage, and depend on the insect species.[129] Within a single leaf, the leaf area that displayed a reduced photosynthetic rate in response to herbivory was six times larger than that removed by feeding.[190] The neglect of photosynthesis in insect–plant nutritional studies presents a serious constraint of gravimetry. Consequently, little is known about the dynamics of energy flow in insect–plant interactions under ecologically relevant conditions. Significant advances in this field will have to rely on the application of a combination of several techniques. Infrared gas analysis (IRGA) can be combined with gravimetric methods to minimize inherent random and systematic errors. IRGA measurements should be combined with the doubly labelled water method for longer term *in planta* quantification



1/3 = ECI   1/2 = ECD   2/3 = AD

Figure 5.8 Partitioning of ingested food (**3**) between the fractions assimilated (**2**) and excreta (faeces) and the subsequent partitioning between growth (**1**) and respiration (CO_2 and H_2O). Commonly used formulae to calculate utilization efficiencies (nutritional indices: ECI, ECD, and AD) from the different fractions (**1**, **2**, and **3**) are given.

of metabolic rates on actively photosynthesizing plant tissues.[174]

Performance (i.e. the extent to which a herbivore is able to realize maximum growth and reproduction) is preferentially expressed as a rate parameter. The most commonly used parameter is the *relative growth rate* (RGR), expressed as the amount of growth attained (mg dry matter) per unit of bodyweight (mg dry matter) per unit of time. Relative growth rate is the product of *relative consumption rate* (RCR, mg per mg per day) and nutritional indices: $\mathrm{RGR} = \mathrm{RCR} \times \mathrm{AD} \times \mathrm{ECD} = \mathrm{RCR} \times \mathrm{ECI}$.

This equation indicates that, on a certain food source, a higher RGR can be attained either by increasing food intake rate or by increasing utilization. When consumption or growth rates are related to gravimetrically determined nutritional indices, inverse relationships are commonly found.[81,147,164] However, such relationships do not allow a distinction between cause and consequence: is growth rate reduced because of a lower metabolic efficiency or is metabolic efficiency reduced because of a lower growth rate? The growth rate of herbivorous insects is assumed to be nutrient limited rather than limited by energy constraints (see Section 5.1.1).[143,162,175,183] Thus, suboptimal availability of a limiting nutrient, often nitrogen or water, reduces growth rate, increases maintenance costs, and produces a lower metabolic efficiency. Intake of the limiting nutrient can, however, be increased by compensatory feeding responses, that, as discussed below, is now known to be well developed in various herbivorous insects.[156] When the ingested amount of a nutrient varies, quantitative effects on the utilization efficiency of that nutrient can result. When utilization efficiency changes as the result of some other factor (toxic allelochemicals, other nutrients, temperature, etc.), the change is due to a qualitative effect. Quantitative and qualitative effects may interact. Employing utilization plots allows distinctions to be made between quantitative, qualitative, and interactive effects (see also Section 5.3.4).[134]

(c) Utilization of plant food by different feeding guilds

When looking at performance and utilization values, large differences appear to exist between different feeding guilds, such as mandibulate feeders of herbs and forbs versus woody plants, or mandibulate versus haustellate (piercing–sucking) species (Table 5.2).

Tree-feeding species realize a much lower RGR, due to both a lower RCR and a lower AD. Haustellate species reach the highest RGR at an RCR that on average is twice as low as that of mandibulates; this can be ascribed to higher values of both AD and ECD. The differences in nutritional indices can be explained largely by the differences in nutritional quality of the respective tissues exploited by these guilds. Thus, piercing–sucking insects grow, on average, faster than leaf-chewers. Obviously such differences are likely to have important ecological consequences.

(d) Changes in food utilization during development

Digestive performance values change during growth. Values for approximate digestibility (AD;

Table 5.2 Average values (range in parentheses) of performance and indices of nutritional utilization by mandibulate and haustellate herbivorous insects (data from Slansky and Scriber, 1985)[164]

	AD (%)	**ECD (%)**	**RCR (mg per mg per day)**	**RGR (mg per mg per day)**	***N***
Mandibulates (Lepidoptera)					
Herbs	53	41	2.0 (0.27–6.0)	0.37 (0.03–1.5)	26
Grasses	43	45	2.0 (0.07–4.8)	0.29 (0.06–0.62)	6
Trees	39	37	1.5 (0.31–5.0)	0.17 (0.03–0.51)	82
Haustellates (Homoptera)					
Herbs	60	65	1.0 (0.90–1.6)	0.39 (0.11–0.67)	3

Note: AD, approximate digestibility; ECD, efficiency of conversion of digested food; RCR, relative consumption rate; RGR, relative growth rate; *N*, no. of insect species investigated.

see Section 5.3.2b) tend to decrease from early to late instars.[6,164] This is probably related to increased feeding rate and increased gut size when larvae grow. Shorter retention times and larger food masses would make enzymatic degradation and nutrient absorption through the gut wall less efficient. Obviously, this may have important consequences for an insect's ability to utilize a particular plant or plant part. It may explain why young insects are often more finicky eaters than older conspecifics. First-instar larvae of *Helicoverpa virescens*, for instance, show a reduction in weight gain due to the presence of condensed tannins in their food at a concentration about 10 times lower than that required to reduce growth of fifth instars.[121]

The fact that food utilization efficiencies are typically higher for early-instar compared with late-instar larvae, together with the observation that levels of detoxifying enzymes are much lower in earlier than in later instars,[2,31] seems of crucial importance when investigating the suitability of a plant for a given insect species. Because variations in nutritive as well as secondary components of the food may have their greatest impact on early-instar larvae, studies on nutritive requirements and effects of allelochemicals should begin with early-instar larvae, in spite of the technical difficulties this may present.[164]

(e) The cost of growth: factors determining metabolic efficiency

A relevant physiological question concerning host-plant utilization is how differences in utilization efficiency come about. The *metabolic load* or *physiological efficiency hypothesis* says that increased energetic processing costs (to be distinguished from maintenance costs) are a direct cause of lower growth rates, suggesting a trade-off between energy production for a range of metabolic processes on the one hand and anabolism, which is the principal process resulting in growth, on the other. This idea has been put forward repeatedly, but experimental evidence is lacking.[135,158,164] Induction of the polysubstrate mono-oxygenases (PSMOs) enzyme system in response to allelochemicals (see Section 5.3.4) in the food has been one of the supposedly more important energy-requiring processes in the metabolic load hypothesis. However, an experimental test that was set up to quantify gravimetrically the cost of this induction did not yield proof for this, and the amount of enzymic PSMO protein measured, although effective in detoxification, was too small to expect any measurable cost.[122]

Few data are available on direct, longer-term measurements of metabolism, for example by respirometry, and it is premature to draw a reliable conclusion on the effect of dietary quality on processing costs.[174] Owing to the laboriousness of such measurements,[171] few studies are available in which repeated or chronic respirometric measurements are combined with determinations of dry matter growth, allowing an actual check of the gravimetric budget.[172] Migratory locusts (*Locusta migratoria*), a hemi-metabolous species, have distinctly lower growth rates than caterpillars (e.g. larvae of *Pieris brassicae*; Table 5.3) and clearly invest more energy per unit of growth. The values of cost of growth for these two species, defined as the ratio between heat loss to the environment and growth (ratio H/G; Table 5.3), differ by only a factor of 1.5, in spite of the considerable differences in total amount of growth achieved, growth rate,

Table 5.3 Costs of growth for a holometabolous and a hemimetabolous insect species in their final larval stages for which continuous or repeated respirometric data as well as gravimetric growth data are available (data from van Loon, 1991, 1993)[172,173]

Species	Diet	Duration (h)	G (mg)	RGR (mg per mg per day)	H (J)	H/G (J/mg)
Pieris brassicae	Cabbage leaves	90	88	0.640	1027	11.7
Locusta migratoria	Wheat	240	258	0.124	4536	17.6

Note: Feeding took place on an optimal host plant. G, growth (mg dry matter per mg per day); RGR, relative growth rate; H, heat production, calculated from respirometric measurements.

body size, and lifestyle between the two. A similar comparison between two other species, a grasshopper (*Melanoplus sanguinipes*) and a caterpillar (*Pseudaletia unipuncta*), when feeding on the same host plant (wheat) under identical conditions, reached the same conclusion: here, also, overall oxygen consumption required to double body mass was twice as high for the grasshopper.[26] An important part of the higher energy costs associated with the growth of the grasshopper results from extended duration of development *per se*, which is expected to result in a greater contribution of maintenance energy to total energy expenditure. An important fraction of the higher maintenance costs for the orthopterans is devoted to their cuticle, the mass of which is 10 times greater than in caterpillars of similar size. From these physiological considerations it emerges that the large degree of variation in ECD reported for herbivorous insects based on gravimetric measurements is unlikely, especially in cases in which growth rates were hardly or not affected.[174] In fact, for ectotherms belonging to different animal classes, spanning several orders of magnitude of body size, the cost of growth is remarkably similar (7–9 J/mg dry bodyweight) when measured along metabolic techniques.[128]

The majority of studies in insect–plant nutritional ecology deal with larval insects that display sedentary habits on their food plant and spend little time and energy on locomotion. Metabolic rate has been observed to increase temporarily during feeding (by a factor of 1.5–5 for caterpillars)[3,102,110] and by 3–4 for locust nymphs.[70] Increased respiration rate in caterpillars has been ascribed to the muscular activity of feeding activity or to an increase in digestive activity following feeding.[3,110] However, in locusts the calorigenic effect of feeding seems not to be associated with energetic costs of digestion and absorption, and has been ascribed to a state of neural arousal.[70] To what extent these transient increases in metabolic rate contribute to total metabolic expenditure during an entire developmental phase has not been studied.

Carnivores such as predators and parasitoids are nutritionally in a more comfortable position than herbivores, because the composition of their food closely fulfils their requirements for growth and development. As a result the approximate digestibility (AD) of animal tissue is higher than that of vegetative food. Whereas folivorous insects show AD values in the range of 40–50% (see Table 5.2), carnivores generally reach values around 80%.[164] Nevertheless, vegetarians may grow faster as they have access to unlimited amounts of food. As a result their relative growth rate (RGR), although quite variable, is usually high, as exemplified for some groups in Table 5.2. The values reached by herbivores (between 0.03 and 0.40) are in marked contrast to the values known for carnivorous species, which range between 0.01 and 0.03. Rapid growth combined with low energy expenditure on food acquisition means that herbivorous insects pass twice as much of their assimilated food to the production of body tissues and eggs than predatory species (Fig. 5.9).

5.3.3 Suboptimal food and compensatory feeding behaviour

(a) Extent and mechanisms of compensatory feeding

As mentioned above, herbivorous insects are faced with a food supply that is not very nutritious. Moreover, plant chemical composition can vary greatly in time and space. To overcome these difficulties many herbivores compensate for suboptimal foods by increasing food intake and/or altering their diets in order to maximize growth rates. Several mechanisms may be used.[92] First, they can leave a nutritionally poor plant that was selected previously to feed upon, and start searching for alternative food. This behaviour is essentially based on a nutritional feedback, whereby the insect resumes food-plant selection and may feed alternately from different host species.[156] This phenomenon, which can be studied in an experimental design employing a 'cafeteria' setup, has been called 'dietary self-selection' (see Section 8.6.2).[177]

Second, herbivores can increase food ingestion rate on the same plant, as mentioned above. This compensatory behaviour counteracts a reduced growth rate and concomitant higher maintenance costs, and may well be a functional response, assuming that the added costs of a higher feeding rate are smaller (see Section. 5.3.2.e).[110] Compensatory

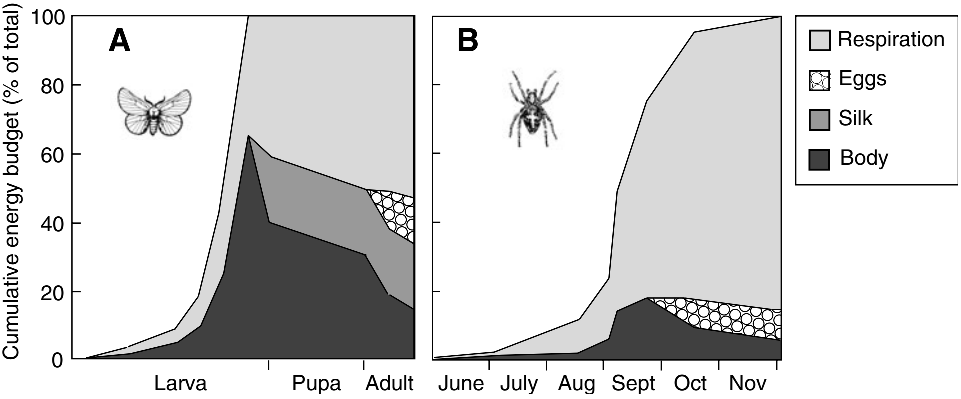


Figure 5.9 Cumulative energy budgets of (**A**) a herbivorous insect (silkworm, *Bombyx mori*) and (**B**) a predatory arthropod (a spider, *Oligolephus tridens*). Energy expenditure as a percentage of total assimilated energy is shown for the lifetime of a female. Note the differential allocation of energy that goes to cover maintenance metabolism compared with that stored in growth. The greater respiratory energy demands of the spider are related to hunting activity. ((**A**) Redrawn from Hiratsuka, 1920;[84] (**B**) redrawn from Klekowski and Duncan, 1975.[97])

feeding by adjusting feeding rate to approach or realize maximum growth rate has been found in a number of cases and is probably general among herbivorous insects.[156] When food quality is suboptimal and protein is the limiting nutrient for growth, food consumption rate has been found to increase to 2.5–3-fold.[164,165] An example is provided by larvae of the monarch butterfly (*Danaus plexippus*). When their host plants, milkweed, were grown on a low nitrogen fertilization scheme, their leaf nitrogen content was only 2.5% N (dry wt) compared with 6.4% N for plants grown at high nitrogen fertilization levels. Monarch larvae feeding on low nitrogen treatment leaves consumed per unit of time almost twice (on a leaf fresh-weight basis) the amounts eaten by larvae feeding on high-treatment leaves.[104]

Third, the insect can, at least in theory, optimize utilization efficiencies, keeping consumption rate constant. Because of the probably spurious interactions between rates and efficiencies,[172] very few convincing data are available for the latter option.

In experiments using artificial diets that allow levels of specific components to be diluted, consumption rates increased by a factor of 7 have been noted (reviewed by Simpson and Simpson[156]). The physiological mechanisms allowing such a considerable span of variation in food consumption rates are complicated.[105,156] Recently, models have been formulated to integrate the various mechanosensory as well as peripheral and internal chemosensory feedbacks that operate in concert,[154] but a discussion of this is beyond the scope of this text.

The speed with which these feedbacks can operate is remarkable. One particularly well studied case involved *Locusta migratoria*. An injection into the haemocoel of a mixture of eight amino acids suffices to postpone the next meal significantly, suggesting that some as yet unknown internal chemosensors monitor haemolymph composition and provide a feedback response on feeding behaviour within minutes.[1] In this insect, compensatory self-selection was also found to occur after only one nutritionally inadequate meal had been taken.[157]

(b) Constraints on compensatory feeding

Although several laboratory studies demonstrate that compensatory feeding can alleviate the effects of nutritional inadequacy of food sources, there are apparent constraints of both a physiological and an ecological nature. First, a trade-off probably exists between rate and efficiency. An increase in rate of consumption leads to a reduced retention time of food in the gut and this in turn will result in lower absorption efficiency.[156] Second, as protein and carbohydrate intake in the locust, for example, are regulated separately,[153] an increased consumption rate to compensate for suboptimal availability of one nutrient may lead to an excess and thereby reduced utilization of the other, partly counteracting the effect of compensation.[191] Third, increased consumption may cause intoxication because of the concomitantly increased ingestion of allelochemicals: the detoxification system cannot keep up with the increased speed at which allelochemicals enter the body.[165] Herbivores may prevent intoxication, however, if they possess peripheral chemoreceptors that detect such allelochemicals (see Chapter 7), or by avoiding such food sources through 'aversion learning' (see Chapter 8).

Regarding ecological trade-offs, it has been hypothesized that in herbivores short feeding periods will be selected for in order to expose the insects minimally to natural enemies.[162,170] Indeed, dramatic differences in selection pressure on feeding versus resting insects have been demonstrated in a study of the predation risks of caterpillars under field conditions. During feeding, the risk of being predated was as much as 100 times greater than during non-feeding periods.[27] Indirect evidence comes from studies in which reduced nitrogen contents of a crop increased predation rates of cabbage white caterpillars (*Pieris rapae*).[109] This may be explained by assuming that compensatory feeding, known to counteract suboptimal nitrogen ingestion rates in these caterpillars,[162] led to increased exposure times and consequently to an increased likelihood of predation.

5.3.4 Allelochemicals and food utilization

Allelochemicals can negatively affect the nutritional physiology of herbivores in three ways:

1. They can reduce food intake by an inhibitory effect on feeding behaviour (see Chapter 7).
2. Once ingested, they can reduce the efficiency of food utilization.
3. They can poison the insect by interference with vital metabolic processes.

Frequently, allelochemicals act through a combination of all three mechanisms. The various postingestive modes of action, which may operate in the gut or, after being absorbed, within other body parts, are often hard to separate.[32,74,160] Here, we discuss some effects on food utilization, because allelochemicals figure as one of the main factors in plant resistance by reducing growth and development of the herbivore. Allelochemicals often interfere with the intrinsic nutritional value of a plant in 'non-adapted' insects, which therefore cannot grow on that plant species or cultivar. Allelochemicals probably also affect food utilization, although obviously to a lesser degree, in insect species that have adopted the plant as a normal host. This may explain why a number of insects fare better on artificial diets than on their natural host plants.

When food utilization indices on an artificial diet supplemented with allelochemicals are compared with control values, negative (or positive) effects of the additive can be quantified. In such a study with a polyphagous insect, five secondary plant compounds, when added to the diet, were all found to suppress growth, even when no reduction of food intake occurred. Different compounds had different effects on the various utilization parameters, suggesting that they interfere with different aspects of the digestion/absorption process.[18] These results, although indicative, need to be repeated with modern rigid protocols in view of their important implications.[141] Utilization plots provide useful insights also in this type of study (see Fig. 5.10). When the migratory locust *Locusta migratoria* was exposed to diets containing varying levels of tannic acid, the effect of this allelochemical depended on the protein : carbohydrate (P : C) ratio. At low P : C ratio food intake rate was reduced, whereas at high P : C ratio (an excess of protein) nitrogen utilization efficiency was reduced. This can be derived graphically from the regression lines in utilization plots which have distinctly different slopes (representing nitrogen utilization efficiency) on diets differing in their P : C ratios (Fig. 5.10).[155] Transgenic plants lacking particular allelochemicals may in the near future also provide useful tools for analysing postingestive detrimental effects of these compounds in adapted insects.

Biochemical approaches can supply more detailed insight into the mode of action of particular secondary plant substances. Gossypol (**28**), a sesquiterpenoid typically occurring in cotton, inhibits feeding and growth in many insects. Larvae of *Spodoptera littoralis*, for instance, grow much faster on cotton leaves of low gossypol

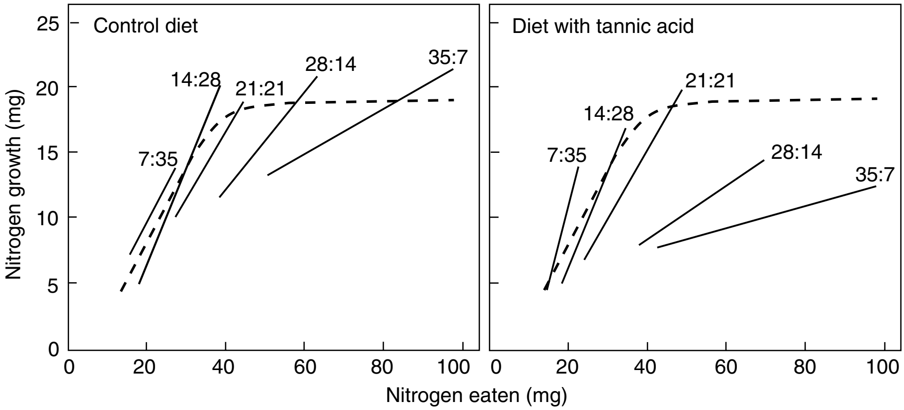


Figure 5.10 Relationships between nitrogen converted to growth and nitrogen eaten by *Locusta migratoria* over its fifth instar on diets varying in protein:carbohydrate (P:C) ratio (7:35, 14:28, 21:21, 28:14, 35:7) and the effect of added tannic acid (at 3.3, 6.7, or 10% levels). Segments of regression lines based on data from 96 insects. The dashed line in both panels shows the expected relationship between nitrogen eaten and nitrogen growth over the whole set of P:C ratios, obtained by fitting through the linear segments indicated in the panel for the control diet (diet free of tannic acid). Addition of tannic acid to diets with a low P:C ratio caused the animals to consume less due to the feeding deterrency of tannic acid, although the slope of the relationship between intake and growth (the higher the slope, the higher nitrogen conversion efficiency) remained unchanged, indicating a quantitative effect of the allelochemical. However, when the diet contained a high P:C ratio, at similar protein consumption (protein levels equal 6.25 times nitrogen levels) conversion to protein growth was decreased (smaller slope); this can be regarded as a qualitative effect of the allelochemical. (Courtesy of S.J. Simpson; redrawn from data in Simpson and Raubenheimer, 1996.)[154]

content than on high-gossypol strains. The proof that it is indeed gossypol that retards growth comes from an experiment with artificial diets. When larvae were fed gossypol-incorporated diets, protease and amylase activities in the gut decreased within 1 day (Table 5.4). The affinity of gossypol to proteins in the gastrointestinal tract is well established. It may bind to the ingested dietary proteins, or to the digestive enzymes themselves. In both cases protein digestion will be hampered.[115]

Another component in the multi-mechanism defence system found in many plant species is the presence of both constitutively and damage-induced protease inhibitors. These compounds form stable complexes with digestive proteolytic enzymes in the insect gut, thereby reducing the release of amino acids from ingested proteins. Insects may, however, counter the negative effects of such protease inhibitors by altering the composition of their midgut proteases by inducing enzymes that are insensitive to the introduced inhibitor, but have the same mode of action as those found in the absence of the plant protease inhibitor. Such is the case, for example, in larvae of *Helicoverpa armigera*, a polyphagous species that possesses a large number of protease-encoding genes responsible for the production of enzymes with differing properties for protein digestion. These genes may be upregulated in response to the protease inhibitors in a particular food plant, which as a result lose their detrimental effects on food utilization.[34]

Table 5.4 Protease activity in 90–100-mg larvae of *Spodoptera littoralis* fed for 2 days on an artificial diet containing various amounts of gossypol acetate (modified from Meisner *et al.*, 1978)[115]

Gossypol acetate concentration (%)	Average larval weight after 2 days (mg)	Protease activity relative to control (%)
0 (control)	546	100
0.25	491	89
0.50	392	55

Interactions between secondary plant compounds and nutrients have been inferred from tests in which the nutritional content was varied, in combination with varying amounts of a particular allelochemical. Such an experiment (Fig. 5.11) showed that the deleterious effect of rutin (**53**), a widely distributed flavonoid, varies not only with

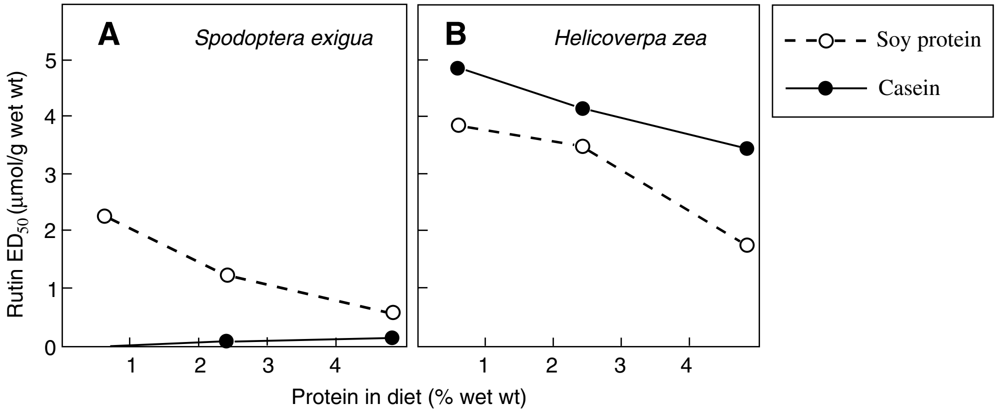


Figure 5.11 Effect of different regimens of dietary protein on the ability of rutin (**53**) to suppress larval growth in two noctuid species. Growth inhibition at various dietary regimens, as expressed by the dose of rutin required to reduce growth by 50% (ED_{50}), is relative to the growth of control insects at 2.4% casein. Note that in *H. zea* growth on casein diet is suppressed at lower rutin concentrations than on soy protein diet, whereas in *S. exigua* the effect of protein type is reversed. (From Duffey *et al.*, 1986.)[59]

the amount of protein in the food, but also with the kind of protein.[59]

Feeny's classic paper[62] on the differential growth of winter moth larvae on young and mature oak leaves (see Fig. 10.4) initiated a lengthy debate on the role of tannins as digestibility reducers. Feeny suggested that tannins form complexes either with leaf protein or with digestive enzymes in the gut, thereby reducing the efficiency of digestion and, as a consequence, retarding growth.[62] Although the affinity of tannins for proteins is probably to some extent responsible for part of the detrimental effects, alternative mechanisms have come to light, such as inhibition of feeding, induction of midgut lesions, and pharmacological toxicity.[14,30,63] The biochemical basis for the antinutritional effects of tannins seems complex and has not yet been fully elucidated.

Insects that are adapted to tannin-rich food are unaffected by, and may even benefit from, the presence of tannins in their food by stimulation of ingestion, among others factors (see Fig. 2.4).[93] The tree locust *Anacridium melanorhodon* shows increased dry matter digestibility (AD) and growth efficiency (ECD), and a resultant 15% increase in growth rate, when tannic acid is added to its diet.[30] Tannin-adapted insects possess several mechanisms to avoid the potentially harmful effects of tannins, including an alkaline gut pH[9] and absorption of tannins on to the peritrophic membrane.[30,62] Others have a polymerization mechanism in their gut[80] or concentrate and egest the polyphenols in their diet.[99]

In Chapter 4 it was stated that plants never contain only one resistance compound, but rather produce a whole chemist's shelf of chemicals. Plant chemicals may in various ways interact synergistically once they are inside the insect body.[20] As known from experience with synthetic insecticides, insects can readily develop resistance to a specific group of chemicals. There is ample evidence that developing resistance to two or more groups with different modes of action is much more difficult. Consequently, adaptation to high levels of toxins in one host plant is often associated with a concomitant reduction in tolerance to compounds in other host plants.[69] In view of these insect response characteristics, a plant probably cannot afford to produce only a single secondary chemical or even a single group of chemicals. In line with this reasoning, wild parsnip produces secondary compounds from at least seven distinct biosynthetic pathways. The toxicity or deterrency of one group of compounds may be strongly affected by the presence or absence of other compounds.[20] Myristicin (**39**) is a lignan with a functional group characteristic of inhibitors of pivotal detoxification enzymes (cytochrome P450). It commonly co-occurs with the phototoxic furanocoumarins in

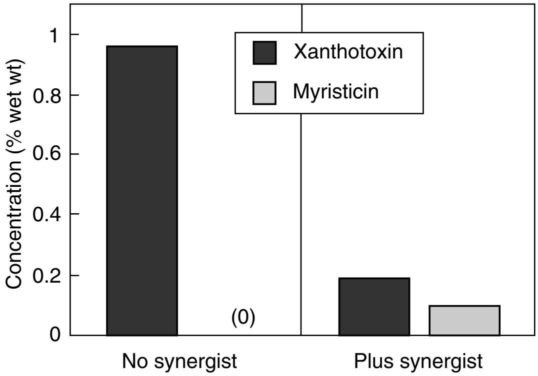


Figure 5.12 Concentration (% wet wt) of xanthotoxin (a furanocoumarin found in umbelliferous plants) which, when added to artificial diets in combination with myristicin (co-occurring in umbellifers and a synergist of organic insecticides) or alone, results in a 50% mortality rate for first-instar larvae of *Helicoverpa zea*. (From Berenbaum and Neal, 1985.)[24]

umbelliferous plants. Myristicin synergizes the toxicity of xanthocoumarin to the generalist caterpillar *Helicoverpa zea* almost 5-fold. Thus, the production of 1 mg of myristicin can 'save' the plant producing 77 mg of furanocoumarin (Fig. 5.12). This may represent a marked saving in production costs (energy), a characteristic of an adaptive strategy.[24] Undoubtedly, synergistic interactions between allelochemicals constitute an extremely important element in a plant's chemical protection. The fact that the number of documented cases of synergy among plant compounds is still limited is probably a result of the difficulty associated with detection and analysis of such interactions. More statistically sophisticated approaches are now available that may stimulate further research on this important chemo-ecological phenomenon.[60,123]

5.3.5 Detoxification of plant allelochemicals

Herbivores are confronted with relatively large amounts of noxious chemicals in their plant food and thus expose themselves to the hazard of being poisoned by every meal, as aptly stated in a founding paper by Brattsten.[35] These potentially toxic compounds can be tolerated because herbivorous species have evolved various physiological mechanisms to avoid their harmful effects. They may either rapidly excrete the unwanted compounds or degrade them enzymatically, or otherwise neutralize such chemicals before they can reach pharmacologically active levels. As a last resort they have developed target-site insensitivity, that is failure of a toxicant to bind to the target because of an alteration in the structure or accessibility of that target site.[21] As insect herbivores consume huge amounts of food relative to their bodyweight (a caterpillar may eat five times its bodyweight per day), their detoxification system needs to be highly efficient. Indeed, insects seem to exhibit a greater tolerance to, for instance, hydrogen cyanide (HCN) and alkaloids. HCN, toxic to all higher organisms, is released from plant cyanogens during digestion (see Section 4.6). Interestingly, polyphagous insects tend to be more than 100 times less sensitive to HCN than mammals (Table 5.5). Likewise, the toxicity of alkaloids to unspecialized insect species appears to be one or two orders of magnitude lower than for mammals (Fig. 5.13).

Table 5.5 Oral toxicity of hydrogen cyanide in some polyphagous insect herbivores compared with mammals (data from Bernays, 1982)[25]

Animals	Oral LD_{50} (mg/kg)
Locusta migratoria (Orthoptera)	500
Zonocerus variegatus (Orthoptera)	1000
Spodoptera littoralis (Lepidoptera)	800
Spodoptera eridania (Lepidoptera)	1500
Mammals (general)	0.5–3.5

Note: LD_{50}, dose at which 50% of animals died.

Specialized insects are often able to cope with still higher concentrations of the allelochemicals that typically occur in their food plants.[25] However, a comparison based on bodyweight is disputable. When toxicity is compared on the basis of metabolic activity or body surface, rather than on bodyweight, the differences between mammals and insects become less spectacular, or may even disappear.

(a) Physiological adaptations: rapid excretion

An effective way to prevent poisoning consists of mechanisms that render target sites inaccessible. This can be accomplished by preventing potential toxins from passing through the gut wall. Under physiological conditions, many secondary plant compounds are either charged molecules, bulky

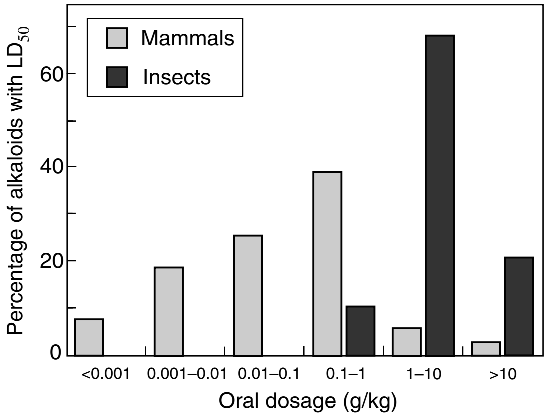


Figure 5.13 Alkaloid toxicity levels for unspecialized mammal herbivores and unspecialized insect herbivores, shown as the percentage of alkaloids with the LD_{50} in the different dosage ranges. (From Bernays, 1982.)[25]

or polar molecules, or hydrophilic compounds (such as glycosides), to which biomembranes are often almost impermeable. Such properties, in combination with rapid intestinal passage normal for herbivorous insects, mean that many toxicants get little chance to enter the body cavity.[185]

A striking example is found in the tobacco hornworm *Manduca sexta*, which feeds on hosts containing nicotine, a traditional insecticide and deadly poison to other animals as well. Nicotine (**41**), in contrast to many other alkaloids, is lipophilic and therefore readily passes the gut epithelium in most insects. It derives its toxicity by means of a functional resemblance to acetylcholine, a pivotal neurotransmitter in the central nervous system of animals. By mimicking acetylcholine molecules, nicotine disturbs delicate and basic functions of the central nervous system. Tobacco hornworm larvae have evolved a number of resistance mechanisms that protect them against this otherwise potent toxin. They rapidly excrete nicotine and other ingested alkaloids before a toxic dose can accumulate. In an experiment in which hornworms were fed food containing known quantities of nicotine, 93% of an ingested 0.5-mg dose was excreted in 2 h, whereas in houseflies more than 90% of the administered dose remained in the insect's body for as long as 18 h.[149] The small amounts of nicotine that do get into the haemolymph of tobacco hornworms cannot cross the ion-impermeable neural sheath and are eliminated via the Malpighian tubules. When, in spite of the physiological barriers that this species has developed, nicotine does reach the nerve cells in the central nervous system, these cells appear to tolerate this compound, demonstrating an example of target-site insensitivity.

This is a well studied example of a multi-component protection system in an insect species adapted against an allelochemical that is highly poisonous to all non-adapted animals. Rapid excretion is not a physiological trait 'invented' only by specialists. The polyphagous caterpillars of the green hairstreak (*Callophrys rubi*), which feed on plants from 10 different families, excrete all alkaloids from their host plant *Genista tinctoria* unchanged. They even do so when raised on a non-host, such as *Lupinus polyphyllus*.[65]

(b) Enzymatic detoxification

Most herbivorous insects rely heavily on enzymatic degradation for neutralization of ingested plant allelochemicals. The most extensively studied enzymes that effectively metabolize a wide variety of toxicants are the cytochrome P450 mono-oxygenases—also called polysubstrate mono-oxygenases (PSMOs) or mixed-function oxydases (MFOs). The P450 enzymes, a diverse class of enzymes found in virtually all insect tissues, play a central role in the metabolism of host-plant chemicals as well as synthesized pesticides by converting them into more polar, reactive compounds, which are further metabolized by secondary enzymes. The P450s derive their name from their spectral absorption maximum around 450 nm. The genome of every insect species carries about 100 P450 genes. This explains the great diversity in the structure of P450 enzymes that forms the basis of their diverse functions in many metabolic pathways.[64]

Activity levels of cytochrome P450 differ greatly among herbivores. In a study of 58 caterpillar species, the activity of this enzyme seemed to be related to the type of food plant. Species feeding on plants rich in monoterpenes, such as members of the Myrtaceae, Rutaceae, or Solanaceae, tend to have considerably higher levels than those living on some other plant families, including Fabaceae, Plantaginaceae, and Poaceae.[139]

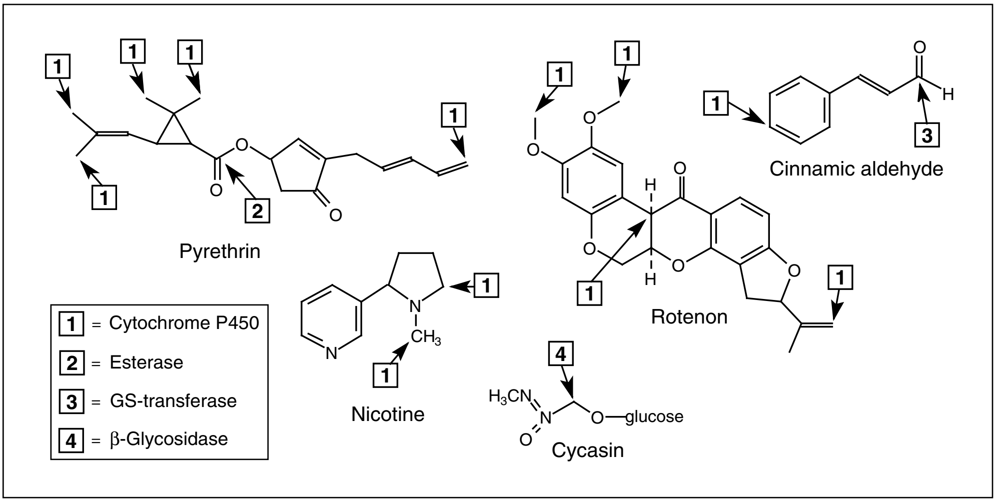


Figure 5.14 Sites of enzymatic attack on some plant allelochemicals. (From Brattsten, 1988.)[36]

Simultaneously with the P450 system, several other enzyme systems serve to detoxify allelochemicals. Toxicants can be metabolized not only by oxidations but also by hydrolytic cleavages and conjugations (Fig. 5.14).

Some types of allelochemical, termed 'pro-oxidants', produce potentially toxic forms of oxygen from interaction with an insect's digestive system. Examples are furanocoumarins, which upon photochemical activation produce both superoxide and singlet oxygen, and hydrolysable tannins, which after oxidation can generate reactive oxygen species. To counteract the toxic effects of oxidants on body tissues and extracellular fluids, herbivorous insects possess a suite of antioxidant enzymes. The amounts of these enzymes vary with type of food and previous exposure to pro-oxidants. Because mature grasses commonly contain small amounts and a limited variety of allelochemicals, graminivorous grasshoppers were expected to have lower levels of antioxidant enzymes in their gut than polyphagous species. A comparison of the concentrations of these enzymes in midgut tissues and the gut fluid of two grasshopper species representing the two feeding habits indeed revealed higher enzyme levels in the polyphagous species.[12,13]

The breakdown products of the toxic compound can be either recycled in the intermediary metabolic pathways or converted to products that are easily excreted. Although enzymatic degradation usually leads to non-toxic products, sometimes a breakdown product is more toxic than the parent molecule (Fig. 5.15). The primary (or phase 1) products are subsequently metabolized by other enzymes to harmless substances.

When insects are exposed to a novel toxin, the levels of detoxifying enzymes, such as P450s, begin to increase within minutes. This phenomenon is termed 'induction' and depends on *de novo* synthesis of enzyme protein by gene activation. Larvae of the noctuid *Peridroma saucia* show low P450 activity when reared on an artificial diet. After being fed peppermint leaves their P450 activity was up to 45 times higher. Enzyme induction was apparently due to high concentrations of monoterpenes in the peppermint leaves, because by feeding artificial diets with menthol or pinene the cytochrome P450 content of the midgut increased considerably.[188] When larvae of the tobacco cutworm *Spodoptera litura*, a polyphagous species, were raised on different hosts belonging to 11 different plant families, their P450 activity levels varied, depending on the kind of food experienced, within a 20-fold range.[139] Moreover, different plants affect reactions controlled by the P450 system differently, a further indication of the existence of isozymes of P450 (Fig. 5.16).[64]

Parsnip webworms (*Depressaria pastinacella*) must contend with many types of furanocoumarin, toxins present in abundance in all of their umbelliferous host plants. In this insect, too, P450 activity

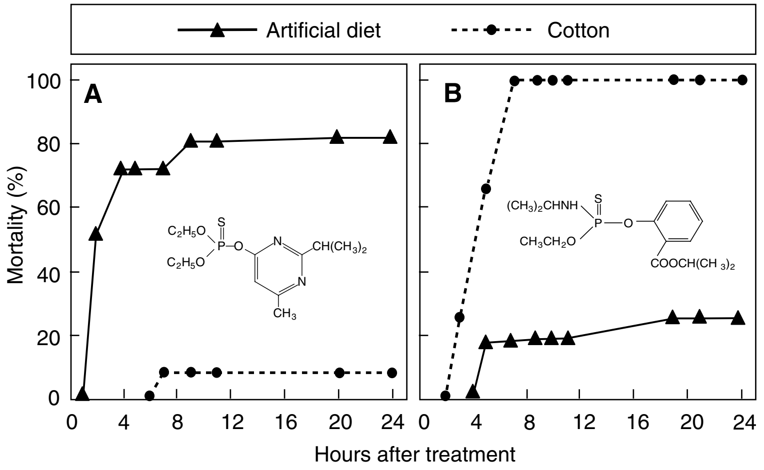


Figure 5.15 Effect of diet on the toxicity of two insecticides. Newly moulted sixth-instar larvae of *Spodoptera frugiperda* were fed artificial diet or cotton leaves for 2 days prior to insecticide treatment. (**A**) 2 μg diazinon per larva. (**B**) 15 μg isofenphos per larva. Rapid breakdown of diazinon in cotton-fed larvae to less toxic compounds reduces mortality. Rapid breakdown of isofenphos in cotton-fed larvae increases mortality, because the resulting metabolite is more toxic than the parent compound. (From Yu, 1986.)[188]

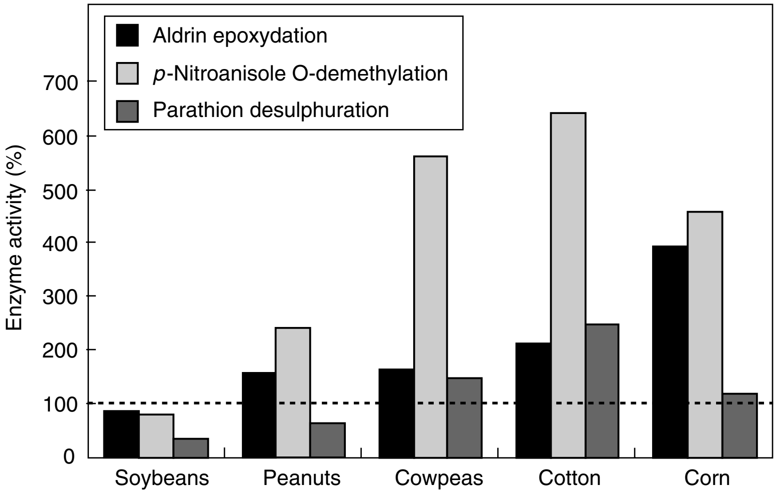


Figure 5.16 Effect of host plant on oxidase activities in the midgut of *Spodoptera frugiperda* larvae. Activities of three different P450 enzymes induced by five different food plants are compared to values measured in larvae fed on an artificial diet (=100%). These data on the selective induction of various P450 activities by different inducers demonstrate the presence of a multiplicity of cytochrome P450 enzymes. (Data from Yu, 1986.)[188]

increases in response to the presence of furanocoumarins in its diet, but in this case no adjustment of their detoxification enzymes to one particular furanocoumarin or to a particular mixture of furanocoumarins could be detected.[45] *Papilio polyxenes* larvae, however, which also feed on umbelliferous plants, possess P450 genes with regulatory elements that appear to respond specifically to particular host-plant allelochemicals, suggesting specificity of regulation.[23]

Activation of P450 defence genes in herbivores in response to plant allelochemicals is an example of phenotypic plasticity, and is obviously of great survival value. The insect's defence strategy shows even more sophisticated details in that it may inflate its P450 enzymes also in response to plant signal compounds, such as jasmonate and salicylate. As discussed in Chapter 4 (see Section 4.14), these compounds are formed in plants after insect (or pathogen) attack and stimulate drastic increases in the levels of defence chemicals. Now, by tuning up their detoxification system upon noticing a plant's signal molecules, insects are physiologically prepared to handle impending rising allelochemical levels in their food.[106]

The phenomenon of inducibility of detoxifying enzymes can explain why a particular insect species shows large differences in sensitivity to insecticide treatment depending on the crop plant species on which it occurs (Fig. 5.16). It also becomes clear why natural enemies of herbivores are commonly more susceptible to insecticide treatments than their hosts: they normally ingest little or no toxin at all with their meals. This applies also to another time-scale, as during evolution parasitoids and predators have not been exposed to the plethora of secondary plant substances as intensively as plant-eating insects.

As soon as the inducing chemical is no longer present, enzyme activity begins to drop to pre-induction levels. Because of this flexible induction mechanism, herbivores can show highly variable enzyme activity levels depending on the food consumed and even on how long after a meal the enzymes were assayed. The phenomenon of induction suggests that there are costs involved in maintaining constantly high levels of detoxifying enzymes. However, there is no evidence that any significant energetic or nutritional costs are involved (see Section 5.3.2).[160] Therefore, the adaptive value of induction remains unclear.

Only by evolving efficient detoxification mechanisms have insects been able to break the chemical protection line of plants—nature's most varied chemical repertoire. These mechanisms have been studied extensively in the recent past and are discussed in several authorative reviews.[37,64,107]

5.4 Symbionts

Life on Earth exists by the mercy of the presence of bacteria. Herbivorous insects, too, are inextricably bound up with the unseen world of microorganisms. When an insect touches a plant, it touches bacteria and their metabolic products. For many herbivores, their capacity to utilize plant food depends on the presence of symbiotic microbes. Evidence that microbes play any role in the digestion and nutrition of folivorous insect species is scarce,[9] but certainly sap-feeding insects can hardly survive without them. Bacteria, yeasts, and other unicellular fungi or protozoa aid in the degradation of plant food and the synthesis of nutritional requisites that plants do not provide at all or provide in insufficient quantities (sterols, some vitamins, 10 of the 20 amino acids).[17,44,131] A third role assigned to symbionts is assistance in the detoxification of plant allelochemicals.

5.4.1 Food utilization and supplementation

Extracellular symbionts live in the alimentary tract, either free in the gut lumen or, more protected, in pockets (caeca) of the midgut or hindgut, as in a number of Coleoptera, Hemiptera, and Orthoptera (Fig. 5.17). Caeca are lacking in Lepidoptera.

Approximately 10% of all insect species accommodate intracellular endosymbionts. They may occur

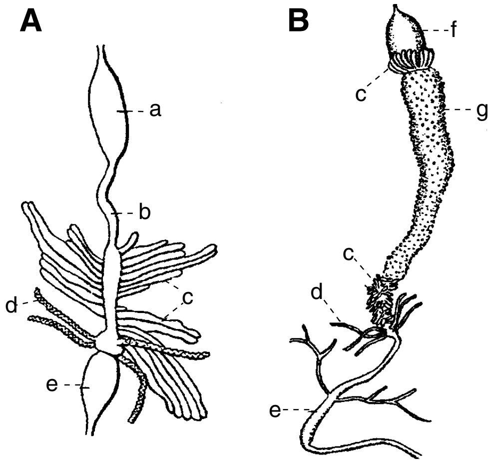


Figure 5.17 Alimentary canals of (**A**) sap-feeding and (**B**) chewing insect species. (**A**) Gut of *Aphanus* sp. (Lygaeidae) feeding on seeds. (**B**) Gut of *Adoxus obscurus* (Chrysomelidae) feeding on *Epilobium angustifolium*. a, dilated part of midgut; b, narrow part of midgut; c, caeca with symbionts; d, Malpighian tubes; e, rectum; f, gizzard; g, midgut.

in cells of the gut wall and be constantly set free into the lumen, as in the larvae of some wood-eating long-horned beetles (Cerambycidae). Often, however, they are confined to specialized cells, *mycetocytes*, which are scattered singly throughout various tissues or are aggregated to form an organ-like structure, called a *mycetome*. Among herbivorous insects the most thoroughly studied groups with respect to endosymbionts are the Hemiptera and Coleoptera. Aphids, for instance, can exploit phloem sap in spite of its nutritional deficiencies because microbial 'brokers' help to overcome the nutritional hurdle.[54] Microorganisms in the gut of the green peach aphid *Myzus persicae* produce all the essential amino acids except for four that are supplied by the host plant.[117] Several other studies on a variety of insect species have demonstrated that symbionts can supplement nutrients that the natural plant food does not provide at all, or that it provides in concentrations too low to support normal growth.[44] Interestingly, recent studies indicate that symbionts can regulate the production or release of individual amino acids in response to the nutritional needs of their host. Thus, these symbionts respond to the well known fact that the amino acid composition of phloem sap varies with plant species and phenology, environmental conditions, and even between different sieve elements of one plant. The notion that *Buchnera* demonstrates a flexibility of production rates of essential amino acids is obviously highly advantageous, if not indispensable, to aphid performance.[57]

5.4.2 Detoxification of plant allelochemicals

Insects that tolerate host plants rich in toxic compounds must possess intrinsic mechanisms to prevent poisoning. In as yet a few cases detoxifying symbionts have been identified as actors in converting host-derived allelochemicals to harmless compounds.[58] The cigarette beetle *Lasioderma serricorne*, for example, houses a yeast that catabolizes a broad variety of xenobiotics, including many flavonoids and tannins. With its biochemical machinery this symbiont most probably contributes to the success of its polyphagous host in exploiting a diverse array of plant species.[150] Another example involving a specialist insect species belonging to a different order underpins the notion that detoxifying symbionts are probably of general occurrence. Apple maggot flies (*Rhagoletis pomonella*) are protected against intoxication by phloridzin (**45**), a flavonoid typical of their host plants. Adult flies obtain nutrients from various sources on leaf surfaces, such as aphid honeydew, microorganisms living on the phylloplane, and foliar leachate containing amino acids, sugars, and also phloridzin. With these substances they ingest bacteria that degradate and detoxify phloridzin in the alimentary tract, whereas flies fed sterilized phloridzin solutions die within 24 h.[103]

In an experimental approach in which germ-free locusts were colonized with specified members of the microbial gut community, several bacteria were found to metabolize secondary plant compounds thereby producing phenolic compounds that are useful to their locust host. Some of these degradation products are selectively antimicrobial and contribute to the suppression of non-indigenous microorganisms, including pathogens. Increasing the diversity of the bacterial gut community increases the effectiveness of this defence system, as was measured when the insect was inoculated with a particular pathogen.[52]

Our hitherto still limited knowledge of the influence of symbionts on food-plant exploitation by insects suggests that they frequently act as unseen but vital mediators in insect–plant interactions.[52] Their importance is underscored by the contention that symbiosis dates back to the early phases of animal evolution and has been a factor leading to the dominance of certain animal clades. Thus, the bacterium *Buchnera aphidicola*, the symbiont of aphids, has co-evolved with hosts for 200 million years, based on a long-term pattern of strict co-speciation.[55,119] This notion may remind us once more that insect-plant associations have tight connections with many other forms of organismal life.

5.5 Host-plant quality affected by microorganisms

Mutualistic or plant pathogenic microorganisms can bring about changes in plant chemistry that may affect insect herbivores sharing the same plant, and hence the two may interact indirectly.

5.5.1 Plant pathogens

Plant diseases probably occur equally commonly in both natural and agricultural ecosystems. As diseased plants are, in addition to having changed physical characteristics, biochemically different from healthy plants, their nutritional suitability for herbivores will be changed. In diseased plants the concentrations and distribution of assimilates (sugars, amino acids, starch, etc.) and allelochemicals are often significantly modified.[16]

Insect herbivores are often negatively affected when their host plant is infested by some phytopathogen, although positive effects have been reported too.[16,76] Two examples may suffice. The chrysomelid beetle *Gastrophysa viridula*, a specialized feeder on dock (*Rumex* spp.), shows greater larval mortality, retarded development, and reduced fecundity on plants infected by rust fungus compared with healthy plants. Chemical analysis showed that infected leaves had lower nitrogen levels and higher oxalate concentrations than rust-free plants, and this probably accounts for the deleterious effects. The interactions in this tripartite system, however, are bilateral, as not only did the insects suffer from the presence of rusts but the beetles, by damaging their food plant, also elicited an induced resistance against the rust fungus. This plant response developed rapidly in the damaged leaf and was also transferred, albeit to a limited degree, to undamaged plant parts.[77]

A striking example of improved food-plant quality for an aphid caused by a plant pathogen is found in fungus-infected silver birch trees (*Betula pendula*). Aphids (*Euceraphis betulae*) not only showed a clear preference for fungus-infected leaves over asymptomatic leaves, but also displayed higher population growth rate and enhanced embryo development. Aphid performance was positively correlated with some leaf chemistry parameters, in particular the free amino acid concentration (Table 5.6). The changes due to fungal infection reflect a physiological response of the plant similar to that occurring during leaf senescence. The resulting altered leaf chemistry presumably forms the mechanistic basis for improved aphid performance.[90]

Table 5.6 Leaf chemistry of *Betula pendula* foliage and aphid performance on asymptomatic leaves (AL) and fungus-infected leaves (FIL) (data from Johnson *et al*., 2003)[90]

	AL	FIL
Leaf constituents		
Phenolic concentration	100	128
Free amino acid concentration	100	239
Aphid performance		
Adult mass	100	114
No. of adults with developed embryos	100	136
No. of embryos in adults with developed embryos	100	127

Note: All values are presented as percentages of the value for asymptomatic leaves.

Virus-infected plants have occasionally been found to be a better food source for insects than healthy plants. Insects that vector plant diseases (homopterans, thrips, mites, beetles), and through infection modify their host plants to their own benefit, in fact optimize resource exploitation. Such is the case with Mexican bean beetles (*Epilachna varivestis*), which prefer to feed on virus-infected bean (*Phaseolus vulgaris*) plants over healthy plants. Their larvae also grow faster on virus-infected leaf tissue. Apparently a mutually beneficial relationship exists between the beetle and the viruses that it vectors. The virus benefits from being transmitted and the insect benefits from better larval growth on diseased plant tissue. Chemical changes in the plant inflicted by the phytopathogen most likely bring about the increased insect performance.[120]

5.5.2 Endophytic fungi

Changes in a plant's chemical composition resulting from the presence of so-called endophytic fungi have in several cases been found markedly to affect herbivore performance. Associations between endophytic fungi and various plants are classified as mutualistic, as these fungi have limited or no pathogenic effects, but may rather provide protection against herbivores (Fig. 5.18) and plant pathogens. The contribution of such endophytes to the greater vegetative vigour of their hosts consists mainly of the ability to produce alkaloids or other compounds that predispose their hosts against

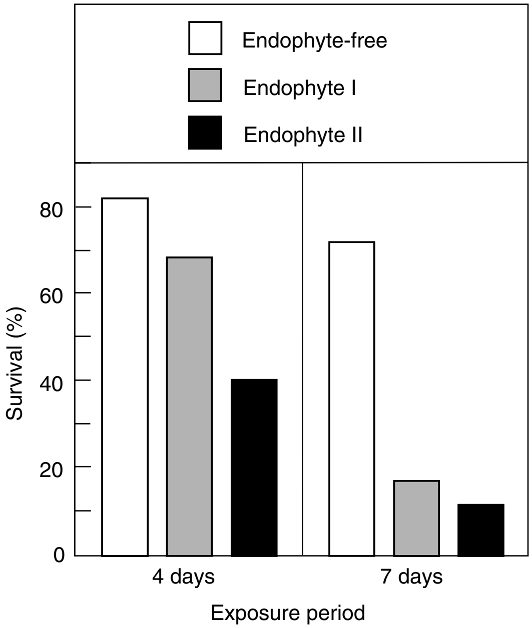


Figure 5.18 Survival of chinch bugs (*Blissus leucopterus hirtus*) on uninfected and endophyte-inoculated Chewings fescue turfgrass (*Festuca rubra*). The two endophyte types were obtained from different grasses. (From Yue *et al.*, 2000.)[189]

herbivory. Fungal endophytes (defined as fungi capable of symptomless occupation of apparently healthy plant tissue) occur in a wide range of grasses and are therefore of agricultural importance. This has stimulated a multitude of papers in recent years.[38,47] As many grasses are relatively free of defensive chemicals, the evolutionary *raison d'être* of endophytic fungi may lie in the mutualistic relationship they have established with their hosts. Especially domesticated grasses often show very high levels of endophyte infection, whereas natural grass populations usually consist of mosaics of infected and uninfected plants. This difference is one of the reasons that the defensive role of endophytes in natural settings has recently been questioned.[61]

Fungal endophytes are not limited to grasses. There is increasing evidence that they are associated with many more angiosperms,[169] including woody plants,[140] than hitherto known. When tomato plants were infected with the unspecialized, widespread, soil-borne fungal endophyte *Acremonium strictum*, larval mortality and developmental time in the polyphagous larvae of *Helicoverpa armigera* were increased in comparison to uninfected plants. In this case the negative effects could not be ascribed to the presence of alkaloids or deterrent compounds in the food plant, but were caused by other fungus-mediated physiological changes in the plant that affected the herbivore's food utilization.[89a]

A host of recent studies indicates that our current thinking about insect–plant relationships needs to be expanded to the broader perspective of multitrophic interactions. As an interesting example, the multitrophic system involving larvae of the Japanese beetle *Popillia japonica*, feeding on the roots of fescue grasses, is given. When host plants of this insect were infected by endophytic fungi, larval food intake was reduced because of the presence of feeding-deterrent alkaloids. As a result larval vigour was lowered, rendering them in turn more susceptible to entomopathogenic nematodes.[71] Whereas this study was restricted to the influence of an endophyte–plant association upon a single herbivore and its pathogen, the impacts of microbial symbionts may be expected to affect many more members of the food web to which the plant belongs. Thus, a comparison of an aphid–parasite food web, naturally assembled on plots of endophyte-infected Italian ryegrass (*Lolium multiflorum*), showed that different aphid species responded differently to the presence or absence of endophytes. Moreover, their parasitoid–hyperparasitoid complexes were also significantly altered, indicating multitrophic consequences of the presence of mutualistic microorganisms in their host plant.[43,124]

Endophytic fungi do not always confer herbivore resistance to their host, as some studies have shown that plant–endophyte interactions even benefit herbivores.[169] However, despite some contrasting results, the growing body of literature suggests that these endophytic fungi play an appreciable role in the nature and strength of plant–herbivore interactions.

The information on the role of microorganisms in detoxification of secondary metabolites of plants and in the modification of plant chemistry shows that insect–plant interactions are not isolated from interactions between plants and other organisms. This is further explained in Chapter 10.

5.6 Host-plant effects on herbivore susceptibility to pathogens and insecticides

A compelling body of evidence indicates that the food plant may influence an insect's susceptibility to entomopathogens such as bacteria,[9,121] viruses,[144] fungi,[75] and nematodes.[15] Effects ascribed to the plant can be either inhibition of the pathogen or potentiation of its toxicity and reproduction. Assuming a decisive role for allelochemicals in interactions between trophic levels, most studies of this phenomenon have focused on the effects of plant compounds on entomopathogen efficacy. Such studies either related the concentration of certain allelochemicals in host plants with pathogenicity, or analysed the effects of pure compounds by adding them in conjunction with the pathogen to artificial diets. By employing the latter method, rutin (**53**) was found markedly to protect within a certain concentration range the larvae of *Trichoplusia ni* against the toxin produced by *Bacillus thuringiensis*.[101] Several classes of plant allelochemical are now known to influence pathogens or the toxins they produce, but other foliar factors such as nutritional value, age, and water content may be involved as well. Gypsy moth larvae show differences in susceptibility to a baculovirus depending on the kind of tree foliage they were offered before and during the test. After inoculation with a standard dose of the virus, the mortality rate on foliage with low levels of hydrolysable tannins is higher than that on high-tannin foliage (Table 5.7).[94] Because larval mortality is also correlated with differences in leaf tissue acidity (which affects the pH of the insect's midgut) the interaction between host plant and pathogen susceptibility of the herbivore may be multifactorial (see also Section 10.4).[95]

Greenhouse whiteflies (*Trialeurodes vaporariorum*), another polyphagous insect, show striking differences in susceptibility to the entomopathogenic fungus *Beauveria bassiana*, depending on its food plant (Fig. 5.19). In contrast to baculoviruses, this pathogen invades its host actively through the cuticle. The lower susceptibility to this fungus when feeding on tomato plants may be due to the presence of tomatine in its cuticle or hemolymph, as this compound is known for its antifungal quality.[132]

Table 5.7 Host-plant effects on mortality of gypsy moth (*Lymantria dispar*) larvae fed standard doses of a baculovirus (from Keating *et al.*, 1988)[94]

Host plant	Mortality (%)	Hydrolysable tannins (% dry weight of leaf)
Black oak (*Quercus nigra*)	25	33.2
Red oak (*Q. rubra*)	47	36.6
Quaking aspen (*Populus tremuloides*)	79	1.4
Bigtooth aspen (*P. grandidentata*)	86	1.2

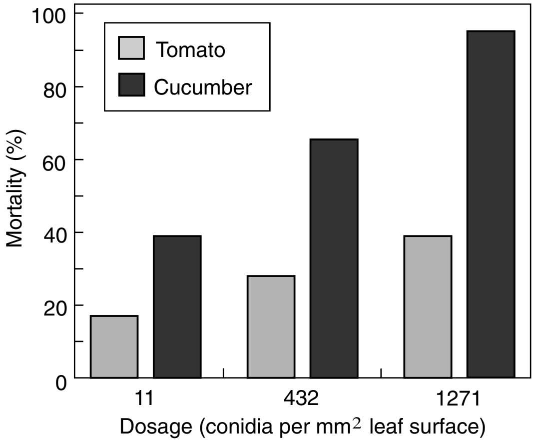


Figure 5.19 Mortality in third instars of greenhouse whitefly (*Trialeurodes vaporariorum*) nymphs after 7 days' rearing on two different host plants inoculated with different dosages of conidia of the entomopathogenic fungus *Beauveria bassiana*. (Data from Poprawski *et al.*, 2000.)[132]

As noted above (Section 5.3), susceptibility to insecticides varies in polyphagous insects with the plant species on which they happen to feed when treated. The migratory grasshopper *Melanoplus sanguinipes* is, when fed oats, killed by a dose of deltamethrin three times lower than when feeding on rye,[83] and aphids (*Myzus persicae*) have shown a 200-fold variation in insecticide susceptibility depending on host plants.[5] Differential insecticide susceptibility has also been linked to physiological variables occurring within one plant species. *Myzus persicae* showed differences in insecticide susceptibility, not only when reared on different varieties of Brussels sprouts, but also on plants of the same cultivar that had been exposed to different nitrogen fertilization regimens. Thus, host-plant condition can also significantly affect the level of insecticide tolerance.[118]

The physiological mechanisms responsible for changes in herbivore sensitivity to pathogens and insecticides remain largely unknown. However, most studies support the general hypothesis that the susceptibility to entomopathogens is inversely related to host-plant suitability. It seems likely that feeding upon a suboptimal host imposes a general stress on the herbivore that negatively influences its resistance to, for instance, microbial infections.[114]

5.7 Food-plant quality in relation to environmental factors

5.7.1 Drought

Water is the means of transport of mineral salts and other materials within the plant. It is drawn through the roots and stem to the leaves where water molecules are split to provide the hydrogen ions (H^+) being used in photosynthesis. Plants also lose water for carbon dioxide at an exchange rate as high as 400 molecules of water per molecule of carbon dioxide fixed. Consequently, plant growth requires large quantities of water.

There are many studies indicating a relationship between a plant's water status and an insect's response, but details of the causal relationships leading from water stress in plants to insect performance are still hardly understood and await elucidation.[73] Drought stress in plants can be extremely detrimental to many herbivores. Sap-feeders in particular are adversely affected by continuous water stress.[87] In other cases, however, effects are negligible or even beneficial for herbivore population increase. Periods of unusually warm, dry weather are often followed by outbreaks of insect pests in forests and rangeland. Their causes are not well understood. Probably drought stress affects the resistance mechanisms of plants negatively, while at the same time their nutritional value for insects increases.[100] Drought, like other kinds of stress, leads to increased levels of soluble sugars and nitrogen in plant foliage, inner bark, and sapwood. Foliar sugars in balsam fir, for instance, may increase 2.5-fold. Despite enhanced foliar nitrogen concentrations, concurrent reductions in turgor and water content interfere with the ability in many herbivores to access or utilize nitrogen.

Drought has also been found to disturb nitrogen metabolism in many woody as well as herbaceous plants, thereby influencing growth and fecundity of their insect fauna.[113] Thus, moisture-deficit stress in plants often induces changes in plant free amino acid accumulations. Proline appears to be the amino acid that reflects water deficit stress most consistently by accumulating to significantly higher levels.[46,113] In the case of cotton, for instance, free proline levels were increased 50-fold in drought-stressed plants.[151] Increased proline (and other free amino acids) levels may increase a plant's susceptibility to insect damage due to the fact that proline acts in many insect species as a feeding stimulant (see Chapter 7).

5.7.2 Air pollution

It has become clear that structural characteristics such as surface morphology and toughness, as well as the levels of both primary metabolites and secondary compounds, can be affected by air pollutants, as clearly outlined in reviews by Hughes[88] and others.[42,79]

Air pollutants considered to be most important in terms of phytotoxicity include sulphur dioxide (SO_2), ozone, nitric oxide (NO), and nitrogen dioxide (NO_2). Evidence of a connection between air pollution and changes in insect attack on plants has been obtained by observational studies (outbreaks of forest insects in the vicinity of industry) and, more recently, by experimental studies. Field studies using controlled release of SO_2 have pointed to extra yield losses attributable to enhanced feeding by, for instance, cereal and conifer aphids.[137] Whereas many aphids and other sap-feeding insect species grow better on plants exposed to moderate concentrations of air pollutants, several species among chewing insects show decreased population densities. Others, however, are definitely favoured by air pollution.

Pollutants may affect herbivore populations by changes in host-plant quality or by affecting their natural enemies. There is a growing amount of evidence that the nutritional quality of plants can be altered significantly. In many instances the levels of free amino acids and reducing sugars are increased, while leaf protein content may either increase or decrease. These changes are reflected in changed nitrogen : carbohydrate ratios.

Contrary to what one would expect, the exposure of host plants to SO_2 often has beneficial effects on herbivore performance.[182] Mexican bean beetles (*Epilachna varivestis*) prefer to feed on soybean foliage that has been exposed to SO_2, and show higher growth rates and increased fecundity on this food. A common change in plant leaves in response to oxidative pollutants, as well as other types of stress, is an increase in the amount of reduced glutathione. In soybean foliage, glutathione concentration was found to change with fumigation in the same manner as insect growth. When non-fumigated foliage was enriched with glutathione by allowing excised leaves to imbibe a solution of this peptide through their petioles, insect growth was stimulated in the same way as by SO_2-treated plants, which suggests a pivotal role for this compound in pollutant-induced effects (Fig. 5.20).[88,89]

Although the evidence for air pollutants affecting plants and thereby insect herbivory is indisputable, our understanding of its consequences for population development or, on a larger scale, the functioning of ecosystems is still close to nil.

Acid precipitation is a phenomenon closely related to air pollution. It is caused primarily by oxides of sulphur and nitrogen. Acid rain probably does not affect plants directly. Most probably, negative influences on plants are caused, especially in soils with poor buffering capacity, by indirect effects through alteration of soil properties and activity of soil microorganisms.[88]

Although carbon dioxide (CO_2) is usually not considered an air pollutant, it is perhaps the most important atmospheric component changed worldwide by human activity. Global concentrations have risen by nearly 30% since the mid-1800s, and a doubling of CO_2 levels is anticipated during the next 50–75 years. Increased CO_2 levels generally affect plant growth and its physical and chemical constitution.[50,127] Most studies on the impact of raised CO_2 levels have shown that in most plants, unaccountably, the carbon to nitrogen ratios in their leaves are increased.[11] To compensate for the lower nutritional quality of their food, several chewing insects were observed to eat more on CO_2-treated plants than on control plants grown in ambient CO_2 levels.[181] In birch trees exposed to twice the normal CO_2 level, the nitrogen concentration of their foliage was decreased by 23%, whereas concentrations were doubled for condensed tannins and tripled for starch. When the foliage was fed to three different lepidopterous insects, the changes in chemical composition caused increased food consumption, unaltered or reduced growth, prolonged larval development, and reduced food-processing efficiency.[108]

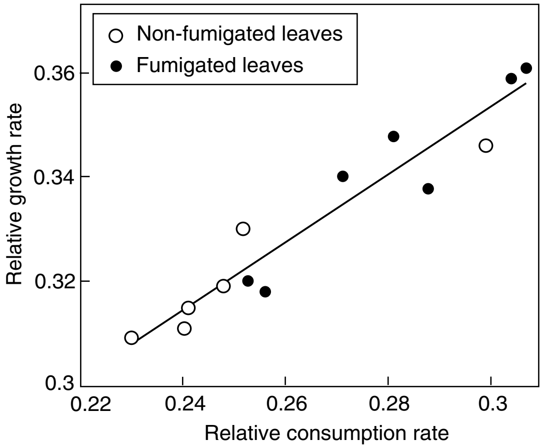


Figure 5.20 Food consumption and growth (expressed as relative consumption and relative growth rates) of Mexican bean beetle larvae fed on previously fumigated soybean leaves (0.3 ppm sulphur dioxide for 24 h) or non-fumigated leaves enriched with glutathione. (Redrawn from Hughes and Voland, 1988.)[89]

The responses of aphids to enhanced CO_2 levels are variable. In some cases aphid performance was increased whereas in other studies negative effects were observed.[86] In an experiment under field conditions, however, densities of aphid natural enemies were much larger under high-CO_2 conditions than under control treatment.[127] Therefore, results from laboratory experiments are of only limited value when used to predict changes in natural communities in response to rising atmospheric CO_2 levels. It should also be realized that other factors that may be influenced by increased

CO_2 concentrations, such as cloud cover, precipitation, and temperature, could easily reverse the direct CO_2 effects on plant–herbivore interactions.[11,178]

5.8 Conclusions

Plant tissue is a far from optimal food source for insects. It is low in nitrogen and high in allelochemicals. Herbivorous insects, with their specific nutritional requirements, are confronted not only with a disparity between their nutritional needs and the chemical composition of their food, but also with another dimension of a living plant, namely an enormous variation in space and time of its nutritional and anti-herbivore components. Foliar monoterpenes in tamarack trees, for instance, exhibit within-tree variations as great as between-tree variations. An individual tree thus appears as a phytochemical mosaic to its insect herbivores.[133] Of course, large within-tree nutritional differences may have great effects on insect performance. Larval growth of the geometrid *Epirrita autumnata* may vary by as much as 30% within a single birch tree. This within-plant heterogeneity in tissue quality is generated largely by sectoriality (the restricted movement of resources along vascular traces within the plant) together with spatial variation in previous damage, nutrient, water, and light availability.[125]

Naturally occurring large variations in plant chemical composition are augmented by environmental factors, such as plant pathogens, air pollution, nutrient and water availability, and other stress factors. Herbivores cope with nutritionally inadequate food by ingesting large amounts of it, a strategy that increases the risk of intoxication by allelochemicals, an example of one of the constraints on compensatory feeding. Effective non-specific and inducible detoxification mechanisms neutralize (to what cost?) toxic food compounds, and symbionts may assist in fulfilling nutritional requirements.

The balance between food quality offered by plants and food quality minimally needed by insects is a subtle one. It is, moreover, affected to a larger extent than hitherto supposed by other partners of an intricate network, such as pathogens and mutualistic organisms.

A large body of literature exists on the quantitative nutritional ecology of insect–plant interactions. However, most of published data are based on laboratory studies that employed gravimetric techniques, using excised plant tissues. Much of the older literature is covered in thorough reviews by Slansky and Scriber[164] and Slansky and Rodriguez.[163] Adoption of innovative combined approaches whereby the metabolic efficiency of a plant-feeding insect on an intact, photosynthetically active plant under relevant ecological conditions can be determined, would mean real progress in this field.[174]

5.9 References

1. Abisgold, J.D. and Simpson, S.J. (1987). The physiology of compensation by locusts for changes in dietary protein. *Journal of Experimental Biology*, **129**, 329–46.
2. Ahmad, S. (1986). Enzymatic adaptations of herbivorous insects and mites to phytochemicals. *Journal of Chemical Ecology*, **12**, 533–60.
3. Aidley, D.J. (1976). Increase in respiratory rate during feeding in larvae of the armyworm, *Spodoptera exempta*. *Physiological Entomology*, **1**, 73–5.
4. Allen, S.E., Grimshaw, H.M., Parkinson, J.A., and Quarmby, C. (1974). *Chemical analysis of ecological materials*. Blackwell, London.
5. Ambrose, H.J. and Regupathy, A. (1992). Influence of host plants on the susceptibility of *Myzus persicae* (Sulz.) to certain insecticides. *Insect Science and its Application*, **13**, 79–86.
6. Anantha Raman, K.V., Magadum, S.B., and Datta, R.K. (1994). Feed efficiency of the silkworm *Bombyx mori* L. hybrid (NB4D2 x KA). *Insect Science and its Application*, **15**, 111–16.
7. Andersen, P.C., Brodbeck, B.V., and Mizell, R.F. (2003). Plant and insect characteristics in response to increasing density of *Homalodisca coagulata* on three host species: a quantification of assimilate extraction. *Entomologia Experimentalis et Applicata*, **107**, 57–68.
8. Anderson, T.E. and Leppla, N.C. (1992). *Advances in insect rearing research and pest management*. Westview Press, Boulder, CO.
9. Appel, H.M. (1994). The chewing herbivore gut lumen: physicochemical conditions and their impact on plant nutrients, allelochemicals, and insect pathogens. In *Insect–plant interactions*, Vol. 5 (ed. E.A. Bernays), pp. 209–23. CRC Press, Boca Raton.
10. Axelsson, B. and Ågren, G.I. (1979). A correction for food respiration in balancing energy budgets. *Entomologia Experimentalis et Applicata*, **25**, 260–6.

11. Ayres, M.P. (1993). Plant defense, herbivory, and climate change. In *Biotic interactions and global change* (ed. P.M. Kareiva, J.G. Kingsolver, and R.B. Huey), pp. 75–94. Sinauer, Sunderland, MA.
12. Barbehenn, R.V. (2003). Antioxidants in grasshoppers: higher levels defend the midgut tissues of a polyphagous species than a graminivorous species. *Journal of Chemical Ecology*, **29**, 683–702.
13. Barbehenn, R.V., Walker, A.C., and Uddin, F. (2003). Antioxidants in the midgut fluids of a tannin-tolerant and a tannin-sensitive caterpillar: effects of seasonal changes in tree leaves. *Journal of Chemical Ecology*, **29**, 1099–116.
14. Barbehenn, R.V. and Martin, M.M. (1994). Tannin sensitivity in larvae of *Malacosoma disstria* (Lepidoptera): roles of the peritrophic envelope and midgut oxidation. *Journal of Chemical Ecology*, **20**, 1985–2001.
15. Barbercheck, M.E., Wang, J., and Hirsh, I.S. (1995). Host plant effects on entomopathogenic nematodes. *Journal of Invertebrate Pathology*, **66**, 169–77.
16. Barbosa, P. (1993). Lepidopteran foraging on plants in agrosystems: constraints and consequences. In *Caterpillars. Ecological and evolutionary constraints on foraging* (ed. N.E. Stamp and T.M. Casey), pp. 523–66. Chapman & Hall, New York.
17. Barbosa, P., Krischik, V.A., and Jones, O.G. (1991). *Microbial mediation of plant–herbivore interactions*. Wiley, New York.
18. Beck, S.D. and Reese, J.C. (1976). Insect–plant interactions: nutrition and metabolism. *Recent Advances in Phytochemistry*, **10**, 41–92.
19. Behmer, S.T. and Nes, W.D. (2003). Insect sterol nutrition and physiology: a global overview. *Advances in Insect Physiology*, **31**, 1–72.
20. Berenbaum, M. (1985). Brementown revisited: interactions among allelochemicals in plants. *Recent Advances in Phytochemistry*, **19**, 139–69.
21. Berenbaum, M.R. (1986). Target site insensitivity in insect–plant interactions. In *Molecular aspects of insect–plant associations* (ed. L.B. Brattsten and S. Ahmad), pp. 257–72. Plenum Press, New York.
22. Berenbaum, M.R. (1995). Turnabout is fair play: secondary roles for primary compounds. *Journal of Chemical Ecology*, **21**, 925–40.
23. Berenbaum, M.R. (2002). Postgenomic chemical ecology: from genetic code to ecological interactions. *Journal of Chemical Ecology*, **28**, 873–96.
24. Berenbaum, M. and Neal, J.J. (1985). Synergism between myristicin and xanthotoxin, a naturally co-occurring plant toxicant. *Journal of Chemical Ecology*, **11**, 1349–58.
25. Bernays, E.A. (1982). The insect on the plant—a closer look. In *Proceedings of the 5th international symposium on insect–plant relationships* (ed. J.H. Visser and A.K. Minks), pp. 3–17. Pudoc, Wageningen.
26. Bernays, E.A. (1986). Evolutionary contrasts in insects: nutritional advantages of holometabolous development. *Physiological Entomology*, **11**, 377–82.
27. Bernays, E.A. (1997). Feeding by lepidopterous larvae is dangerous. *Ecological Entomology*, **22**, 121–3.
28. Bernays, E.A. and Chapman, R.F. (1994). *Host-plant selection by phytophagous insects*. Chapman & Hall, New York.
29. Bernays, E.A. and Lewis, A.C. (1986). The effect of wilting on palatability of plants to *Schistocerca gregaria*, the desert locust. *Oecologia*, **70**, 132–5.
30. Bernays, E.A., Cooper Driver, G., and Bilgener, M. (1989). Herbivores and plant tannins. *Advances in Ecological Research*, **19**, 263–302.
31. Berry, R.E., Yu, S.J., and Terriere, L.C. (1980). Influence of host plants on insecticide metabolism and management of variegated cutworm. *Journal of Economic Entomology*, **73**, 771–4.
32. Blau, P.A., Feeny, P., and Contardo, L. (1978). Allylglucosinolate and herbivorous caterpillars: a contrast in toxicity and tolerance. *Science*, **200**, 1296–8.
33. Blua, M.J., Perring, T.M., and Madore, M.A. (1994). Plant virus-induced changes in aphid population development and temporal fluctuations in plant nutrients. *Journal of Chemical Ecology*, **20**, 691–707.
34. Bown, D.P., Wilkinson, H.S., and Gatehouse, J.A. (2004). Regulation of expression of genes encoding digestive proteases in the gut of a polyphagous lepidopteran larva in response to dietary protease inhibitors. *Physiological Entomology*, **29**, 278–90.
35. Brattsten, L.B. (1979). Biochemical defense mechanisms in herbivores against plant allelochemicals. In *Herbivores. Their interaction with secondary plant metabolites* (ed. G.A. Rosenthal and D.H. Janzen), pp. 200–70. Academic Press, New York.
36. Brattsten, L.B. (1988). Enzymic adaptations in leaf-feeding insects to host-plant allelochemicals. *Journal of Chemical Ecology*, **14**, 1919–39.
37. Brattsten, L.B. (1992). Metabolic defenses against plant allelochemicals. In *Molecular aspects of insect–plant associations* (ed. L.B. Brattsten and S. Ahmad), pp. 176–242. Plenum Press, New York.
38. Breen, J.P. (1994). Acremonium endophyte interactions with enhanced plant resistance to insects. *Annual Review of Entomology*, **39**, 401–23.
39. Brewer, J.W., O'Neill, K.M., and Deshon, R.E. (1987). Effects of artificially altered foliar nitrogen levels on development and survival of young instars of western

spruce budworm, *Choristoneura occidentalis* Freeman. *Journal of Applied Entomology*, **104**, 121–30.

40. Brodbeck, B. and Strong, D. (1987). Amino acid nutrition of herbivorous insects and stress to host plants. In *Insect outbreaks* (ed. P. Barbosa and J.C. Schultz), pp. 347–64. Academic Press, San Diego.
41. Brodbeck, B.V., Mizell, R.F., and Andersen, P.C. (1993). Physiological and behavioral adaptations of three species of leafhoppers in response to the dilute nutrient content of xylem fluid. *Journal of Insect Physiology*, **39**, 73–81.
42. Brown, V.C. (1995). Insect herbivores and gaseous air pollutants—current knowledge and predictions. In *Insects in a changing environment* (ed. R. Harrington and N.E. Stork), pp. 219–49. Academic Press, London.
43. Bultman, T. L., McNeill, M. R., and Goldson, S. L. (2003). Isolate-dependent impacts of fungal endophytes in a multitrophic interaction. *Oikos*, **102**, 491–6.
44. Campbell, B.C. (1989). On the role of microbial symbiotes in herbivorous insects. In *Insect–plant interactions*, Vol. 1 (ed. E.A. Bernays), pp. 1–44. CRC Press, Boca Raton.
45. Cianfrogna, J.A., Zangerl, A.R., and Berenbaum, M.R. (2002). Dietary and developmental influences on induced detoxification in an oligophage. *Journal of Chemical Ecology*, **28**, 1349–64.
46. Claussen, W. (2005). Proline as a measure of stress in tomato plants. *Plant Science*, **168**, 241–8.
47. Clay, K. and Schardl, C. (2002). Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist*, **160**, S99–S127.
48. Cohen, A.C. and Patana, R. (1984). Efficiency of food utilization by *Heliothis zea* (Lepidoptera: Noctuidae) fed artificial diets or green beans. *Canadian Entomologist*, **116**, 139–46.
49. Cohen, A.C. (2004). *Insect diets: science and technology*. CRC Press, Boca Raton, FL.
50. Coley, D., Massa, M., Lovelock, E., and Winter, K. (2002). Effects of elevated CO_2 on foliar chemistry of saplings of nine species of tropical tree. *Oecologia*, **133**, 62–9.
51. Dethier, V.G. and Schoonhoven, L.M. (1968). Evaluation of evaporation by cold and humidity receptors in caterpillars. *Journal of Insect Physiology*, **14**, 1049–54.
52. Dillon, R.J. and Dillon, V.M. (2004). The gut bacteria of insects: nonpathogenic interactions. *Annual Review of Entomology*, **49**, 71–92.
53. Dolezal, P., Bextine, B.R., Dolezalova, R., and Miller, T.A. (2004). Novel methods of monitoring the feeding behavior of *Homalodisca coagulata* (Say) (Hemiptera; Cicadellidae). *Annals of the Entomological Society of America*, **97**, 1055–62.
54. Douglas, A.E. (1998). Nutritional interactions in insect–microbial symbioses: aphids and their symbiotic bacteria *Buchnera. Annual Review of Entomology*, **43**, 17–37.
55. Douglas, A.E. (2003a). *Buchnera* bacteria and other symbionts of aphids. In *Insect symbiosis* (ed. K. Bourtzis and T. Miller), pp. 23–38. CRC Press, Boca Raton.
56. Douglas, A.E. (2003b). The nutritional physiology of aphids. *Advances in Insect Physiology*, **31**, 73–140.
57. Douglas, A.E., Minto, L.B., and Wilkinson, T.L (2001). Quantifying nutrient production by the microbial symbionts in an aphid. *Journal of Experimental Biology*, **204**, 349–58.
58. Dowd, P.F. (1991). Symbiont-mediated detoxification in insect herbivores. In *Microbial mediation of plant–herbivore interactions* (ed. P. Barbosa, V.A. Krischik, and O.G. Jones), pp. 411–40. Wiley, New York.
59. Duffey, S.S., Bloem, K.A., and Campbell, B.C. (1986). Consequences of sequestration of plant natural products in plant–insect–parasitoid interactions. In *Interactions of plant resistance and parasitoids and predators of insects* (ed. D.J. Boethel and R.D. Eikenbary), pp. 31–60. Ellis Horwood, Chichester.
60. Duke, J.A. and Bogenschutz-Godwin, M.J. (1999). The synergy principle at work in plants, pathogens, insects, herbivores, and humans. In *Natural products from plants* (ed. P.B. Kaufman, L.J. Cseke, S. Warber, J.A. Duke, and H.L. Brielmann), pp. 183–205. CRC Press, Boca Raton.
61. Faeth, S. H. (2002). Are endophytic fungi defensive plant mutualists? *Oikos*, **98**, 25–36.
62. Feeny, P. (1970). Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology*, **51**, 565–81.
63. Feeny, P. (1992). The evolution of chemical ecology: contributions from the study of herbivorous insects. In *Herbivores. Their interactions with secondary plant metabolites*, Vol. 2 (2nd edn) (ed. G.A. Rosenthal and M.R. Berenbaum), pp. 1–44. Academic Press, San Diego.
64. Feyereisen, R. (1999). Insect P450 enzymes. *Annual Review of Entomology*, **44**, 507–33.
65. Fiedler, K., Krug, E., and Proksch, P. (1993). Complete elimination of hostplant quinolizidine alkaloids by larvae of a polyphagous lycaenid butterfly *Callophrys rubi. Oecologia*, **94**, 441–5.
66. Fischer, K. and Fiedler, K. (2000). Response of the copper butterfly *Lycaena tityrus* to increased leaf nitrogen in natural food plants: evidence against the nitrogen limitation hypothesis. *Oecologia*, **124**, 235–41.

67. Forkner, R.E. and Hunter, M.D. (2000). What goes up must come down? Nutrient addition and predation pressure on oak herbivores. *Ecology*, **81**, 1588–1600.
68. Gelman, D.B., Bell, R.A., Liska, L.J., and Hu, J.S. (2001). Artificial diets for rearing the Colorado potato beetle, *Leptinotarsa decemlineata*. *Journal of Insect Science*, **1**(7), 1–11.
69. Gould, F. (1991). Arthropod behavior and the efficacy of plant protectants. *Annual Review of Entomology*, **36**, 305–30.
70. Gouveia, S.M., Simpson, S.J., Raubenheimer, D., and Zanotto, F.P. (2000). Patterns of respiration in *Locusta migratoria* nymphs when feeding. *Physiological Entomology*, **25**, 88–93.
71. Grewal, S.K., Grewal, P.S., and Gaugler, R. (1995). Endophytes of fescue grasses enhance susceptibility of *Popillia japonica* larvae to an entomopathogenic nematode. *Entomologia Experimentalis et Applicata*, **74**, 219–24.
72. Guthrie, W.D., Larvis, J.L., and Reed, G.L. (1984). Leaf-feeding damage by European corn borer (Lepidoptera: Pyralidae) larvae reared one generation each year on dent maize and eight generations each year on a meridic diet (for eight years) and then reared continuously on a meridic diet for eight additional years. *Journal of the Kansas Entomological Society*, **57**, 352–4.
73. Haile, F.J. (2001). Drought stress, insects, and yield loss. In *Biotic stress and yield loss* (ed. R.K.D. Peterson and L.G. Higley), pp. 117–34. CRC Press, Boca Raton.
74. Hägele, B.F. and Rowell-Rahier, M. (1999). Dietary mixing in three generalist herbivores: nutrient complementation or toxin dilution. *Oecologia*, **119**, 521–33.
75. Hajek, A.E. and St Leger, R.J. (1994). Interactions between fungal pathogens and insect hosts. *Annual Review of Entomology*, **39**, 293–322.
76. Hammond, A.M. and Hardy, T.N. (1988). Quality of diseased plants as hosts for insects. In *Plant stress–insect interactions* (ed. E.A. Heinrichs), pp. 381–432. John Wiley, New York.
77. Hatcher, P.E. (1995). 3-Way interactions between plant–pathogenic fungi, herbivorous insects and their host plants. *Biological Reviews of the Cambridge Philosophical Society*, **70**, 639–94.
78. Haukioja, E., Ossipov, V., and Lempa, K. (2002). Interactive effects of leaf maturation and phenolics on consumption and growth of a geometrid moth. *Entomologia Experimentalis et Applicata*, **104**, 125–36.
79. Heliövaara, K. and Väisänen, R. (1993). *Insects and pollution*. CRC Press, Boca Raton.
80. Henn, M. (1999). The changes of polyphenols as a result of the passage through the gut of the gypsy moth *Lymantria dispar* (Lep., Lymantriidae): influence on the growth of the larvae. *Journal of Applied Entomology*, **123**, 391–5.
81. Henn, M.W. and Schopf, R. (2001). Response of beech (*Fagus sylvatica*) to elevated CO_2 and N: influence on larval performance of the gypsy moth *Lymantria dispar* (Lep., Lymantriidae). *Journal of Applied Entomology*, **125**, 501–5.
82. Henriksson, J., Haukioja, E., Ossipov, V., Ossipova, S., Sillanpää, S., Kapari, L., *et al*. (2003). Effects of host shading on consumption and growth of the geometrid *Epirrita autumnata*: interactive roles of water, primary and secondary compounds. *Oikos*, **103**, 3–16.
83. Hinks, C.F. and Spurr, D.T. (1989). Effect of food plants on the susceptibility of the migratory grasshopper (Orthoptera: Acrididae) to deltamethrin and dimethoate. *Journal of Economic Entomology*, **82**, 721–6.
84. Hiratsuka, E. (1920). Researches on the nutrition of the silkworm. *Bulletin of the Sericulture Experimental Station Japan*, **1**, 257–315.
85. Hirayama, C., Konno, K., and Shinbo, H. (1996). Utilization of ammonia as a nitrogen source in the silkworm, *Bombyx mori*. *Journal of Insect Physiology*, **42**, 983–8.
86. Holopainen, J.K. (2002). Aphid response to elevated ozone and CO_2. *Entomologia Experimentalis et Applicata*, **104**, 137–42.
87. Huberty, A.F. and Denno, R.F. (2004). Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology*, **85**, 1383–98.
88. Hughes, P.R. (1988). Insect populations on host plants subjected to air pollution. In *Plant stress–insect interactions* (ed. E.A. Heinrichs), pp. 249–319. John Wiley, New York.
89. Hughes, P.R. and Voland, M.L. (1988). Increase in feeding stimulants as the primary mechanism by which SO_2 enhances performance of Mexican bean beetle on soybean. *Entomologia Experimentalis et Applicata*, **48**, 257–62.

89a. Jallow, M.F.A., Dugassa-Gobena, D., and Vidal, S. (2004). Indirect interaction between an unspecialized endophytic fungus and a polyphagous moth. *Basic and Applied Ecology*, **5**, 183–91.

90. Johnson, S.N., Douglas, A.E., Woodward, S., and Hartley, S.E. (2003). Microbial impacts on plant–herbivore interactions: the indirect effects of a birch pathogen on a birch aphid. *Oecologia*, **134**, 388–96.
91. Kam, M. and Degen, A.A. (1997). Energy budget in free-living animals: a novel approach based on the doubly labelled water method. *American Journal of Physiology*, **272**, 1336–43.

92. Karban, R. and Agrawal, A.A. (2002). Herbivore offense. *Annual Review of Ecology and Systematics*, **33**, 641–64.
93. Karowe, D.N. (1989). Differential effect of tannic acid on two tree-feeding Lepidoptera: implications for theories of plant anti-herbivore chemistry. *Oecologia*, **80**, 507–12.
94. Keating, S.T., Yendol, W.G., and Schultz, J.C. (1988). Relationship between susceptibility of gypsy moth larvae (Lepidoptera; Lymantriidae) to a baculovirus and host plant foliage constituents. *Environmental Entomology*, **17**, 952–8.
95. Keating, S.T., Schultz, J.C., and Yendol, W.G. (1990). The effect of diet on gypsy moth (*Lymantria dispar*) larval midgut pH, and its relationship with larval susceptibility to a baculovirus. *Journal of Invertebrate Pathology*, **56**, 317–26.
96. Keena, M.A., Odell, T.M., and Tanner, J.A. (1995). Effects of diet ingredient source and preparation method on larval development of laboratory-reared gypsy moth (Lepidoptera: Lymantriidae). *Annals of the Entomological Society of America*, **88**, 672–9.
97. Klekowski, R.Z. and Duncan, A. (1975). Physiological approach to ecological energetics. In *Methods for ecological bioenergetics* (ed. W. Grodzinski, R.Z. Klekowski, and A. Duncan), pp. 15–64. Blackwell, Oxford.
98. Kogan, M. (1986). Bioassays for measuring quality of insect food. In *Insect–plant interactions* (ed. J.R. Miller and T.A. Miller), pp. 155–89. Springer, New York.
99. Kopper, B.J., Jakobi, V.N., Osier, T.L., and Lindroth, L.R. (2002). Effects of paper birch condensed tannin on whitemarked tussock moth (Lepidoptera: Lymantriidae) performance. *Environmental Entomology*, **31**, 10–14.
100. Koricheva, J., Larsson, S., and Haukioja, E. (1998). Insect performance on experimentally stressed woody plants: a meta-analysis. *Annual Review of Entomology*, **43**, 195–216.
101. Krischik, V.A. (1991). Specific or generalized plant defense: reciprocal interactions between herbivores and pathogens. In *Microbial mediation of plant–herbivore interactions* (ed. P. Barbosa, V.A. Krischik, and O.G. Jones), pp. 309–340. Wiley, New York.
102. Kukal, O. and Dawson, T.E. (1989). Temperature and food quality influences feeding behavior, assimilation efficiency and growth rate of arctic woolly-bear caterpillars. Oecol*ogia*, **79**, 526–32.
103. Lauzon, C.R., Potter, S.E., and Prokopy, R.J. (2003). Degradation and detoxification of the dihydrochalcone phloridzin by *Enterobacter agglomerans*, a bacterium associated with the apple pest, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). *Environmental Entomology*, **32**, 953–62.
104. Lavoie, B. and Oberhauser, K.S. (2004). Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. *Environmental Entomology*, **33**, 1062–9.
105. Lee, K.P., Raubenheimer, D., and Simpson, S.J. (2004). The effects of nutritional imbalance on compensatory feeding for cellulose-mediated dietary dilution in a generalist caterpillar. *Physiological Entomology*, **29**, 108–17.
106. Li, X., Schuler, M.A., and Berenbaum, M.R. (2002). Jasmonate and salicylate induce expression of herbivore cytochrome P450 genes. *Nature*, **419**, 712–15.
107. Lindroth, R.L. (1991). Differential toxicity of plant allelochemicals to insects: roles of enzymatic detoxification systems. In *Insect–plant interactions*, Vol. 3 (ed. E.A. Bernays), pp. 1–33. CRC Press, Boca Raton.
108. Lindroth, R.L., Arteel, G.E., and Kinney, K.K. (1995). Responses of three saturniid species to paper birch grown under enriched CO_2 atmospheres. *Functional Ecology*, **9**, 306–11.
109. Loader, C. and Damman, H. (1991). Nitrogen content of food plants and vulnerability of *Pieris rapae* to natural enemies. *Ecology*, **72**, 1586–90.
110. McEvoy, P.B. (1984). Increase in respiratory rate during feeding in larvae of the cinnabar moth *Tyria jacobaeae. Physiological Entomology*, **9**, 191–5.
111. McNeill, S. and Southwood, T.R.E. (1978). The role of nitrogen in the development of insect plant relationships. In *Biochemical aspects of plant and animal coevolution* (ed. J.B. Harborne), pp. 77–98. Academic Press, London.
112. Mattson, W.J. (1980). Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, **11**, 119–61.
113. Mattson, W.J. and Haack, R.A. (1979). The role of drought stress in provoking outbreaks of phytophagous insects. In *Insect outbreaks* (ed. P. Barbosa and J.C. Schultz), pp. 365–407. Academic Press, San Diego.
114. Meade, T. and Hare, J.D. (1994). Effects of genetic and environmental host plant variation on the susceptibility of two noctuids to *Bacillus thuringiensis. Entomologia Experimentalis et Applicata*, **70**, 165–78.
115. Meisner, J., Ishaaya, I., Ascher, K.R.S., and Zur, M. (1978). Gossypol inhibits protease and amylase activity of *Spodoptera littoralis* Boisduval larvae. *Annals of the Entomological Society of America*, **71**, 5–8.

116. Mellanby, K. and French, R.A. (1958). The importance of drinking water to larval insects. *Entomologia Experimentalis et Applicata*, **1**, 116–24.
117. Mittler, T.E. (1970). Uptake rates of plant sap and synthetic diet by the aphid *Myzus persicae*. *Annals of the Entomological Society of America*, **63**, 1701–5.
118. Mohamad, B.M. and van Emden, H.F. (1989). Host plant modification to insecticide susceptibility in *Myzus persicae* (Sulz.). *Insect Science and its Application*, **10**, 699–703.
119. Moran, N. (2002). The ubiquitous and varied role of infection in the lives of animals and plants. *American Naturalist*, **160**, S1–S8.
120. Musser, R.O., Hum-Musser, S.M., Felton, G.W., and Gergerich, R.C. (2003). Increased larval growth and preference for virus-infected leaves by the Mexican bean beetle, *Epilachna varivestis* Mulsant, a plant virus vector. *Journal of Insect Behavior*, **16**, 247–56.
121. Navon, A., Hare, J.D., and Federici, B.A. (1993). Interactions among *Heliothis virescens* larvae, cotton condensed tannin and the CryIA(c) δ-endotoxin of *Bacillus thuringiensis*. *Journal of Chemical Ecology*, **19**, 2485–99.
122. Neal, J.J. (1987). Metabolic costs of mixed-function oxidase induction in *Heliothis zea*. *Entomologia Experimentalis et Applicata*, **43**, 175–9.
123. Nelson, A.C. and Kursar, T.A. (1999). Interactions among plant defense compounds: a method for analysis. *Chemoecology*, **9**, 81–92.
124. Omacini, M., Chaneton, E.J., Ghersa, C.M., and Müller, C.B. (2001). Symbiotic fungal endophytes control insect–parasite interaction webs. *Nature*, **409**, 78–81.
125. Orians, C.M. and Jones, C.G. (2001). Plants as resource mosaics: a functional model for predicting patterns of within-plant resource heterogeneity to consumers based on vascular architecture and local environmental variability. *Oikos*, **94**, 493–504.
126. Peng, Z. and Miles, P.W. (1988). Studies on the salivary physiology of plant bugs: function of the catechol oxidase of the rose aphid. *Journal of Insect Physiology*, **11**, 1027–33.
127. Percy, K.E., Awmack, C.S., Lindroth, R.L., Kubiske, M.E., Kopper, B.J., Isebrands, J.G., *et al.* (2002). Altered performance of forest pests under atmospheres enriched by CO_2 and O_3. *Nature*, **420**, 403–7.
128. Peterson, C.C., Walton, B.M., and Bennett, A.F. (1999). Metabolic costs of growth in free-living Garter snakes and the energy budget of ectotherms. *Functional Ecology*, **13**, 500–7.
129. Peterson, R.K.D., Higley, L.G., Haile, J., and Barrigossi, J.A.F. (1998). Mexican bean beetle (Coleoptera: Coccinelidae) injury affects photosynthesis of *Glycine max* and *Phaseolus vulgaris*. *Environmental Entomology*, **27**, 373–81.
130. Petrusewicz, K. and MacFayden, A. (1970). *Productivity of terrestrial animals: principles and methods*, IBP Handbook 13. Blackwell, Oxford.
131. Phelan, P.L. and Stinner, B.R. (1992). Microbial mediation of plant–herbivore ecology. In *Herbivores. Their interactions with secondary plant metabolites*, Vol. 2 (2nd edn) (ed. G.A. Rosenthal and M.R. Berenbaum), pp. 279–315. Academic Press, San Diego.
132. Poprawski, T.J., Greenberg, S.M., and Ciomperlik, M.A. (2000). Effect of host plant on *Beauveria bassiana*- and *Paecilomyces fumosoroseus*-induced mortality of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). *Environmental Entomology*, **29**, 1048–53.
133. Powell, J.S. and Raffa, K.F. (1999). Sources of variation in concentration and composition of foliar monoterpenes in tamarack (*Larix laricina*) seedlings: roles of nutrient availability, time of season, and plant architecture. *Journal of Chemical Ecology*, **25**, 1771–97.
134. Raubenheimer, D. and Simpson, S.J. (1994). An analysis of nutrient budgets. *Functional Ecology*, **8**, 783–91.
135. Rausher, M.D. (1982). Population differentiation in *Euphydras editha* butterflies, larval adaptation to different hosts. *Evolution*, **36**, 581–90.
136. Reese, J.C. and Field, M.D. (1986). Defense against insect attack in susceptible plants: cutworm (Lepidoptera: Noctuidae) growth on corn seedlings and artificial diet. *Annals of the Entomological Society of America*, **79**, 372–6.
137. Riemer, J. and Whittaker, J.B. (1989). Air pollution and insect herbivores: observed interactions and possible mechanisms. In *Insect–plant interactions*, Vol. 1 (ed. E.A. Bernays), pp. 73–105. CRC Press, Boca Raton.
138. Roessingh, P., Bernays, E.A., and Lewis, A.C. (1985). Physiological factors influencing preference for wet and dry food in *Schistocerca gregaria* nymphs. *Entomologia Experimentalis et Applicata*, **37**, 89–94.
139. Rose, H.A. (1985). The relationship between feeding specialization and host plants to aldrin epoxidase activites of midgut homogenates in larval Lepidoptera. *Ecological Entomology*, **10**, 455–67.
140. Saikkonen, N., Helander, M., Ranta, H., Neuvonen, S., Virtanen, T., Suomela, J., *et al.* (1996). Endophyte-mediated interactions between woody plants and insect herbivores? *Entomologia Experimentalis et Applicata*, **80**, 269–71.

141. Schmidt, D.J. and Reese, J.C. (1986). Sources of error in nutritional index studies of insects on artificial diet. *Journal of Insect Physiology*, **32**, 193–8.
142. Schroeder, L.A. (1981). Consumer growth efficiencies, their limits and relationships to ecological energetics. *Journal of Theoretical Biology*, **93**, 805–28.
143. Schroeder, L.A. (1986). Protein limitation of a tree leaf feeding Lepidopteran. *Entomologia Experimentalis et Applicata*, **41**, 115–20.
144. Schultz, J.C. and Keating, S.T. (1991). Host-plant-mediated interactions between the gypsy moth and a baculovirus. In *Microbial mediation of plant–herbivore interactions* (ed. P. Barbosa, V.A. Krischik, and O.G. Jones), pp. 489–506. Wiley, New York.
145. Scott Brown, A.S., Simmonds, M.S.J., and Blaney, W.M. (2002). Relationship between nutritional composition of plant species and infestation levels of thrips. *Journal of Chemical Ecology*, **28**, 2359–409.
146. Scriber, J.M. (1979). Effects of leaf-water supplementation upon post-ingestive nutritional indices of forb-, shrub-, vine-, and tree-feeding Lepidoptera. *Entomologia Experimentalis et Applicata*, **25**, 240–55.
147. Scriber, J.M. (1984). Host-plant suitability. In *Chemical ecology of insects* (ed. W.J. Bell and R.T. Cardé), pp. 159–202. Chapman & Hall, London.
148. Scriber, J.M. and Slansky, F. (1981). The nutritional ecology of immature insects. *Annual Review of Entomology*, **26**, 183–211.
149. Self, L.S., Guthrie, F.E., and Hodgson, E. (1964). Metabolism of nicotine by tobacco-feeding insects. *Nature*, **204**, 300–1.
150. Shen, S.K. and Dowd, P.F. (1991). Detoxification spectrum of the cigarette beetle symbiont *Symbiotaphrina kochii* in culture. *Entomologia Experimentalis et Applicata*, **60**, 51–9.
151. Showler, A.T. (2002). Effects of water deficit stress, shade, weed competition, and kaolin particle film on selected foliar free amino acid accumulations in cotton, *Gossypium hirsutum* (L.). *Journal of Chemical Ecology*, **28**, 631–51.
152. Showler, A.T. and Moran, P.J. (2003). Effects of drought stressed cotton, *Gossypium hirsutum* L., on beet armyworm, *Spodoptera exigua* (Hübner), oviposition, and larval feeding preferences and growth. *Journal of Chemical Ecology*, **29**, 1997–2011.
153. Simpson, S.J. and Abisgold, J.D. (1985). Compensation by locusts for changes in dietary nutrients: behavioural mechanisms. *Physiological Entomology*, **10**, 443–52.
154. Simpson, S.J. and Raubenheimer, D. (1996). Feeding behaviour, sensory physiology and nutritional feedback: a unifying model. *Entomologia Experimentalis et Applicata*, **80**, 55–64.
155. Simpson, S.J. and Raubenheimer, D. (2001). The geometric analysis of nutrient–allelochemical interactions: a case study using locusts. *Ecology*, **82**, 422–39.
156. Simpson, S.J. and Simpson, C.L. (1990). The mechanisms of nutritional compensation by phytophagous insects. In *Insect–plant interactions*, Vol. 2 (ed. E.A. Bernays), pp. 111–160. CRC Press, Boca Raton.
157. Simpson, S.J., Simmonds, M.S.J., and Blaney, W.M. (1988). A comparison of dietary selection behaviour in larval *Locusta migratoria* and *Spodoptera littoralis. Physiological Entomology*, **13**, 225–38.
158. Singer, M. (2001). Determinants of polyphagy by a woolly bear caterpillar: a test of the physiological efficiency hypothesis. *Oikos*, **93**, 194–204.
159. Singh, P. and Moore, R.F. (1985). *Handbook of insect rearing*, 2 Vols. Elsevier, Amsterdam.
160. Slansky, F. (1992). Allelochemical–nutrient interactions in herbivore nutritional ecology. In *Herbivores. Their interactions with secondary plant metabolites*, Vol. 2 (2nd edn) (ed. G.A. Rosenthal and M.R. Berenbaum), pp. 135–74. Academic Press, San Diego.
161. Slansky, F. (1993). Nutritional ecology: the fundamental quest for nutrients. In *Caterpillars. Ecological and evolutionary constraints on foraging*, (ed. N.E. Stamp and T.M. Casey), pp. 29–91. Chapman & Hall, New York.
162. Slansky, F. and Feeny, P. (1977). Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. *Ecological Monographs*, **47**, 209–28.
163. Slansky, F. and Rodriguez, J.G. (1987). *Nutritional ecology of insects, spiders, and related invertebrates*. John Wiley, New York.
164. Slansky, F. and Scriber, J.M. (1985). Food consumption and utilization. In *Comprehensive insect physiology, biochemistry, and pharmacology* (ed. G.A. Kerkut and L.I. Gilbert), Vol. 4, pp. 87–163. Pergamon, New York.
165. Slansky, F. and Wheeler, G.S. (1992). Caterpillars' compensatory feeding response to diluted nutrients leads to toxic allelochemical dose. *Entomologia Experimentalis et Applicata*, **65**, 171–86.
166. Southwood, T.R.E. and Henderson, P.A. (2000). *Ecological methods* (3rd edn). Blackwell Science, Oxford.
167. Spencer, J.L., Mabry, T.R., and Vaughn, T.T. (2003). Use of transgenic plants to measure insect herbivore movement. *Journal of Economic Entomology*, **96**, 1738–49.

168. Stamp, N.E. (1991). Stability of growth and consumption rates and food utilization efficiencies when insects are given an excess of food. *Annals of the Entomological Society of America*, **84**, 58–60.
169. Strauss, S.Y. and Irwin, R.E. (2004). Ecological and evolutionary consequences of multispecies plant–animal interactions. *Annual Review of Ecology, Evolution and Systemtics*, **35**, 435–66.
170. Tabashnik, B.E. and Slansky, F. (1987). Nutritional ecology of forb foliage-chewing insects. In *Nutritional ecology of insects, mites, spiders and related invertebrates* (ed. F. Slansky and J.G. Rodriguez), pp. 71–103. John Wiley, New York.
171. Van Loon, J.J.A. (1988). A flow-through respirometer for leaf chewing insects. *Entomologia Experimentalis et Applicata*, **49**, 265–76.
172. Van Loon, J.J.A. (1991). Measuring food utilization in plant-feeding insects—toward a metabolic and dynamic approach. In *Insect–plant interactions*, Vol. 3 (ed. E.A. Bernays), pp. 79–124. CRC Press, Boca Raton.
173. Van Loon, J.J.A. (1993). Gravimetric vs. respirometric determination of metabolic efficiency in caterpillars of *Pieris brassicae. Entomologia Experimentalis et Applicata*, **67**, 135–42.
174. Van Loon, J.J.A., Casas, J., and Pincebourde, S. (2005). Nutritional ecology of insect–plant interactions: persistent handicaps and the need for innovative approaches. *Oikos*, **108**, 194–201.
175. Van't Hof, H.M. and Martin, M.M. (1989). The effect of diet water content on energy expenditure by third-instar *Manduca sexta* larvae (Lepidoptera, Sphingidae). *Journal of Insect Physiology*, **35**, 433–6.
176. Waldbauer, G.P. (1968). The consumption and utilization of food by insects. *Advances in Insect Physiology*, **5**, 229–88.
177. Waldbauer, G.P. and Friedman, S. (1988). Dietary self-selection by insects. In *Endocrinological frontiers in physiological insect ecology* (ed. F. Sehnal, A. Zabza, and D.L. Denlinger), pp. 403–22. Wroclaw Technical University Press, Wroclaw.
178. Watt, A.D., Whittaker, J.B., Docherty, M., Brooks, G., Lindsay, E., and Salt, D.T. (1995). The impact of elevated atmospheric CO_2 on insect herbivores. In *Insects in a changing environment* (ed. R. Harrington and N.E. Stork), pp. 197–217. Academic Press, London.
179. Welter, S.C. (1989) Arthropod impact on plant gas exchange. In *Insect–plant interactions*, Vol. 1 (ed. E.A. Bernays), pp. 135–150. CRC Press, Boca Raton.
180. White, T.C.R. (1993). *The inadequate environment. Nitrogen and the abundance of animals*. Springer, Berlin.
181. Whittaker, J.B. (1999). Impacts and responses at population level of herbivorous insects to elevated CO_2. *European Journal of Entomology*, **96**, 149–56.
182. Whittaker, J.B. and Warrington, S. (1995). Effects of atmospheric pollutants on interactions between insects and their food plants. In *Pests, pathogens and plant communities* (ed. J.J. Burdon and R.S. Leather), pp. 97–110. Blackwell, Oxford.
183. Wiegert, R.G. and Petersen, C.E. (1983). Energy transfer in insects. *Annual Review of Entomology*, **28**, 455–86.
184. Wightman, J.A. (1981) Why insect energy budgets do not balance. *Oecologia*, **50**, 166–9.
185. Wink, M. and Schneider, D. (1990). Fate of plant-derived secondary metabolites in three moth species (*Syntomis mogadorensis, Syntomeida epilais*, and *Creatonotus transiens*). *Journal of Comparative Physiology B*, **160**, 389–400.
186. Wolf, T.J., Ellington, C.P., Davis, S., and Feltham, M.J. (1996). Validation of the doubly labelled water technique for bumblebees *Bombus terrestris* (L.) *Journal of Experimental Biology*, **199**, 959–72.
187. Woods, H.A. and Bernays, E.A. (2000). Water homeostasis by wild larvae of *Manduca sexta. Physiological Entomology*, **25**, 82–7.
188. Yu, S.J. (1986). Consequences of induction of foreign compound-metabolizing enzymes in insects. In *Molecular aspects of insect–plant associations* (ed. L.B. Brattsten and S. Ahmad), pp. 153–174. Plenum Press, New York.
189. Yue, Q., Johnson-Cicalese, J., Gianfagna, T.J., and Meyer, W.A. (2000). Alkaloid production and chinch bug resistance in endophyte-inoculated chewings and strong creeping red fescues. *Journal of Chemical Ecology*, **26**, 279–92.
190. Zangerl, A.R., Hamilton, J.G., Miller, T.J., Crofts, A.R., Oxborough, K., Berenbaum, M.R., *et al.* (2002). Impact of folivory on photosynthesis is greater than the sum of its holes. *Proceedings of the National Academy of Sciences of the USA*, **99**: 1088–91.
191. Zanotto, F.P., Gouveia, S.M., Simpson, S.J., Raubenheimer, D., and Calder, P.C. (1997). Nutritional homeostasis in locusts: is there a mechanism for increased energy expenditure during carbohydrate overfeeding? *Journal of Experimental Biology*, **200**, 2437–48.

CHAPTER 6

Host-plant selection: how to find a host plant

One of the most notable features of herbivorous insects that has emerged from the previous chapters is that most species are very selective feeders and meticulously choose the plants on which they deposit their eggs. Recent research on several species has shown that they select not only certain plant species but also specific plant organs. At the outset of this chapter on selection behaviour it is important to note that the host-plant range of a certain insect species does not necessarily include all plant species that appear under laboratory testing conditions behaviourally acceptable or nutritionally adequate; under natural circumstances it is often more restricted. Also, host selection behaviour may change with the developmental phase of the insect, and different life stages often differ in their host-plant preference or their ability to use a plant species as a host. Despite the fact that neonate insect larvae have a small body size and consequently possess limited energy reserves, they are capable of leaving the plant on which they hatched if they judge it unsuitable.

There are several situations that make it necessary for an herbivorous insect to search for a host plant. For instance, eclosion of adults from pupae that overwintered in the soil may occur far from potential food or oviposition plants if these are annuals. Arrival in a novel habitat after migration or dispersal, and local exhaustion of food plants, are other examples of such circumstances. In natural habitats, host plants commonly grow together with non-host plants in mixed and complex vegetations. For host-plant specialists, the ability to find and recognize host plants in these habitats is crucial, and this ability constitutes the focus of this and the next chapter.

6.1 Terminology

It is useful first to define terms that are generally used to describe or categorize host-plant selection behaviour.

Searching

Whenever an insect is remote from a potential foodplant, it needs to search for and find that plant. To locate a host plant, the insect needs to move towards it and contact it, or at least to arrive and stay in the proximity of it in order to examine its characteristics further. The observation that the insect contacts the plant, however, gives no information on the mechanism used in establishing this contact. The term 'searching' means 'to look carefully in a place in an effort to find something'. 'Finding' (sometimes unfortunately used as a synonym[104]) may rather be the end result of searching—hence the subtitle of this chapter. As searching has a connotation of directionality, it is important to note that the movement pattern of an insect may vary from random, resulting in contact by chance, to oriented and strongly directed movements (see below).

Selection

In the strict sense of the word, 'to select' means to choose from among alternatives. In order to do this, it is necessary that differential sensory perception of alternative food plants occurs. Selection thus implies a weighing of alternatives. From a methodological point of view, it is difficult to prove that comparison of alternatives is being made during selection behaviour, especially if contacts with potential hosts occur sequentially. Sequential contacting of different host-plant species occurs more frequently than simultaneous contacting and this implies that a short-term memory must be invoked to enable comparisons over time. In cases in which alternatives have been assessed before final acceptance occurs, either at a distance by approaching and turning away again or by actual contact-testing, the term 'selection behaviour' is appropriate.

Acceptance

Acceptance of a plant is said to occur when either sustained feeding or oviposition occurs. 'Acceptance' is a term devoid of the assumptions implied by the term 'selection'. For example, when a beetle is released in the middle of a monoculture of beans and is observed to initiate sustained feeding after climbing a bean plant, it cannot be concluded that the beetle selected the bean plant as a host plant, as no alternatives were available. It can only be said that the bean plant has been accepted by the insect. Acceptance is affected by motivation, the general willingness to feed or oviposit, which itself results from the integration of internal physiological state parameters (e.g. level of satiation, maturation state of eggs) of the insect. Acceptance is a term distinct from acceptability, which is a plant trait and defined as the likelihood that a particular plant species is selected for feeding or oviposition.

Preference

When, in dual or multiple choice assays, an insect consistently feeds or oviposits more often on one of the alternative plants, it is said to 'prefer' that plant over the others. This may also be observed under field conditions when the degree of feeding or oviposition on a certain plant species is higher than would be predicted from its relative abundance. Clearly, preference is a relative concept and applicable only to the set of plant species or genotypes that were actually available to the insect.

Recognition

This term is often used in connection with acceptance. It means 'to know again' and implicitly refers to a neural process. It implies that there is an internal standard or 'image' of the plant(s) sought for. This image is present in one or another form in the central nervous system (CNS) of the insect. The profile of incoming sensory information on plant cues is compared with this stored image and, when it matches sufficiently, the plant is recognized as a host. The putative image is genetically fixed, but can be modified by experience to a fair extent (see Chapter 8).

From the above, it appears that the terms searching, selection, preference, and recognition implicitly refer to complex behavioural processes, the neural mechanisms of which are being elucidated (see below) but as yet are only partly understood. The proper use of these terms is important to avoid confusion between ecologists describing patterns of association and behaviourists addressing mechanisms.[149] Here we use preference as an insect trait that is not influenced by plant density, plant dispersion, or plant quality, unless the insect is learning (see Chapter 8).

It is also important at this point to relate the behavioural terms defined above to the classification of behaviour-modifying chemicals. These chemicals are collectively termed semiochemicals[116] or infochemicals.[50] For this purpose we adopt the terminology proposed by Dethier *et al*.,[48] which is summarized in Table 6.1. Corresponding terms in semiochemical and infochemical terminology are: kairomone, for attractant and feeding and oviposition stimulants; allomone, for repellent and deterrent. Flower volatiles that attract pollinators (see Chapter 12) are examples of synomones.

The difference between semiochemical and infochemical terminology is that, whereas in semiochemical terminology the origin of the produced chemical determines its designation as a kairomone, allomone, or synomone, in infochemical terminology the adaptive value of the use of the information that the chemical carries is the central issue.

Table 6.1 Chemical designations in terms of insect responses (from Dethier *et al*., 1960)[48]

Attractant	A chemical that causes insects to make oriented movements towards its source
Repellent	A chemical that causes insects to make oriented movements away from its source
Arrestant	A chemical that may slow the linear progression of an insect by reducing actual speed of locomotion or by increasing turning rate
Feeding or ovipositional stimulant	A chemical that elicits feeding or oviposition in insects ('feeding stimulant' is synonymous with 'phagostimulant')
Deterrent	A chemical that inhibits feeding or oviposition when present in a place where insects would, in its absence, feed or oviposit

6.2 Host-plant selection: a catenary process

Insects are often said to show 'programmed behaviour' and stereotyped, predictable sequences of behavioural acts—so-called *reaction chains*.[8] This means that more or less distinct behavioural elements follow one another in a fixed order. The insect shows appropriate reactions to a succession of stimuli (Fig. 6.1).

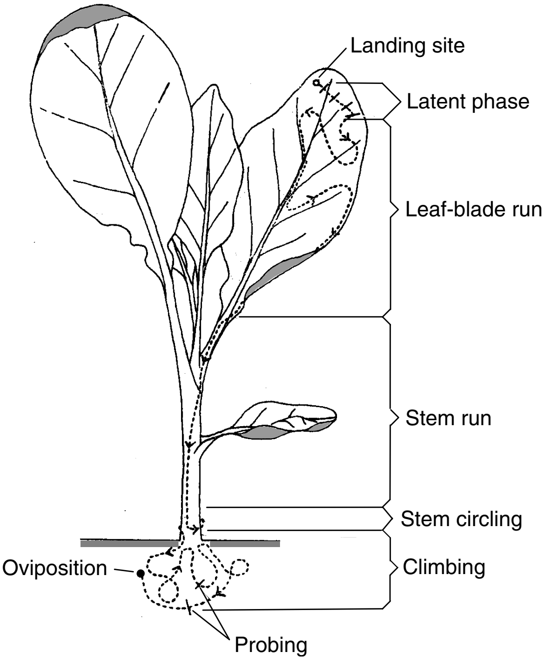


Figure 6.1 Complex behaviour patterns involve a sequence of stimulation and response steps, as exemplified by oviposition behaviour in the cabbage root fly *Delia radicum*. An airborne gravid female fly may land in response to yellow-green wavelengths (500–600 nm), as reflected by green foliage. During the 'latent phase' she walks along the leaf, pausing now and then to groom or to make short flights. During the next phase, the 'leaf-blade run', she walks continuously, often along the leaf edge and frequently changing direction. With taste hairs on her tarsi she assesses the suitability of the plant. If she contacts the appropriate chemical stimuli, she moves on to a midrib of a leaf or a stem, which is quickly followed ('stem run'). At the stem base she moves around it sideways ('stem circling'), keeping her head downwards. During the 'climbing phase' she walks around close to the cabbage stem and occasionally climbs up the stem a few centimetres. She then starts 'probing' the soil with her ovipositor, probably testing soil particle size and water content. When again the adequate stimuli are perceived, she finally lays her eggs in the soil close to the stem. (From Zohren, 1968.)[189]

When the outcome of a sensory evaluation is rejection of a particular plant or plant part as a food or oviposition site, the herbivore 'jumps back' to one of the earlier steps in the reaction sequence. Modification of selection behaviour as a result of previous experience (see Chapter 8) leads to faster decision-making or to changes in preference, but the sequence remains the same. As we will see from the examples presented below, such sequences of behavioural phases and of elements within each phase can be quite long and elaborate.

In the process of host-plant selection two main consecutive phases may be distinguished, delimited by the intermittent decision to stay in contact with the plant: (1) searching and (2) contact-testing. The first phase may end with the event of finding; the second phase ends with acceptance or rejection. Acceptance is a crucial behavioural decision as it results in ingestion of plant material or deposition of eggs, with possible negative consequences for fitness. A host-plant selection sequence is schematically depicted in Figure 6.2A.

Going through the sequence, the number and intensity of the cues that the plant offers to the insect increase, thereby also potentially increasing the intensity and modalities of sensory information that the insect can collect about the plant. A standardized host-plant selection sequence can be described as follows:

1. The insect has no physical contact with a plant and either rests or moves about randomly, walking or flying.
2. It perceives plant-derived cues, optical and/or olfactory.
3. It responds to these cues in such a way that the distance between its body and the plant decreases.
4. The plant is found, i.e. it is contacted by either touching or climbing it, or by landing on it.
5. The plant surface is examined by contact-testing (e.g. palpation of leaf surface).
6. The plant may be damaged and the content of tissues released by nibbling or test-biting (in the case of biting–chewing species), probing (piercing–sucking species), or puncturing with the ovipositor.
7. The plant is accepted (as evidenced by one or more eggs being laid or continued feeding) or is rejected, resulting in the insect's departure.

During each of these steps the insect may decide to turn away from the plant before contacting it, or to leave it after contact. When it arrives in a patch of potential host plants, it may exhibit repetition of the same sequence with respect to different plant individuals of the same or other species. In the end it may return to and select the plant that was examined first but was left after that initial contact.

In this and the next chapters, host-plant selection behaviour will be discussed using this sequential framework. The focus will be on the different plant cues affecting selection behaviour and the sensory apparatus via which these are perceived and affect selection behaviour. The crucial decision to accept or to reject a plant is based not only on sensory information of plant cues but also on the insect's physiological status (satiety, sexual maturity, egg maturation, etc.[13]). The integration of these two variables, together with information on previous experiences stored in the insect's memory, occurs in the CNS.[45] For the purpose of this chapter we will assume that the internal status is such that the insect is not engaged in migration or dispersal activity and that its motivation for feeding or oviposition is high.

It should be noted that not all herbivores follow the standardized sequence described above and summarized in Figure 6.2A. Some take short-cuts and others show more complicated sequences. Some well studied examples have been schematized in Figure 6.2B–E.

6.3 Searching mechanisms

To understand the ways in which herbivorous insects search, it is necessary to present a description of searching behaviour as well as a discussion of the possible causal mechanisms involved.

The sequence of behavioural steps that is passed through during searching differs among insect species and developmental phases, and depends on the cues available. The whole range, varying from random search to highly directed search patterns, has been observed. In the field, random search has been described for various insects, such as polyphagous caterpillars,[46] immature and mature polyphagous locusts,[2,108] and adult oligophagous Colorado potato beetles (*Leptinotarsa decemlineata*).[83]

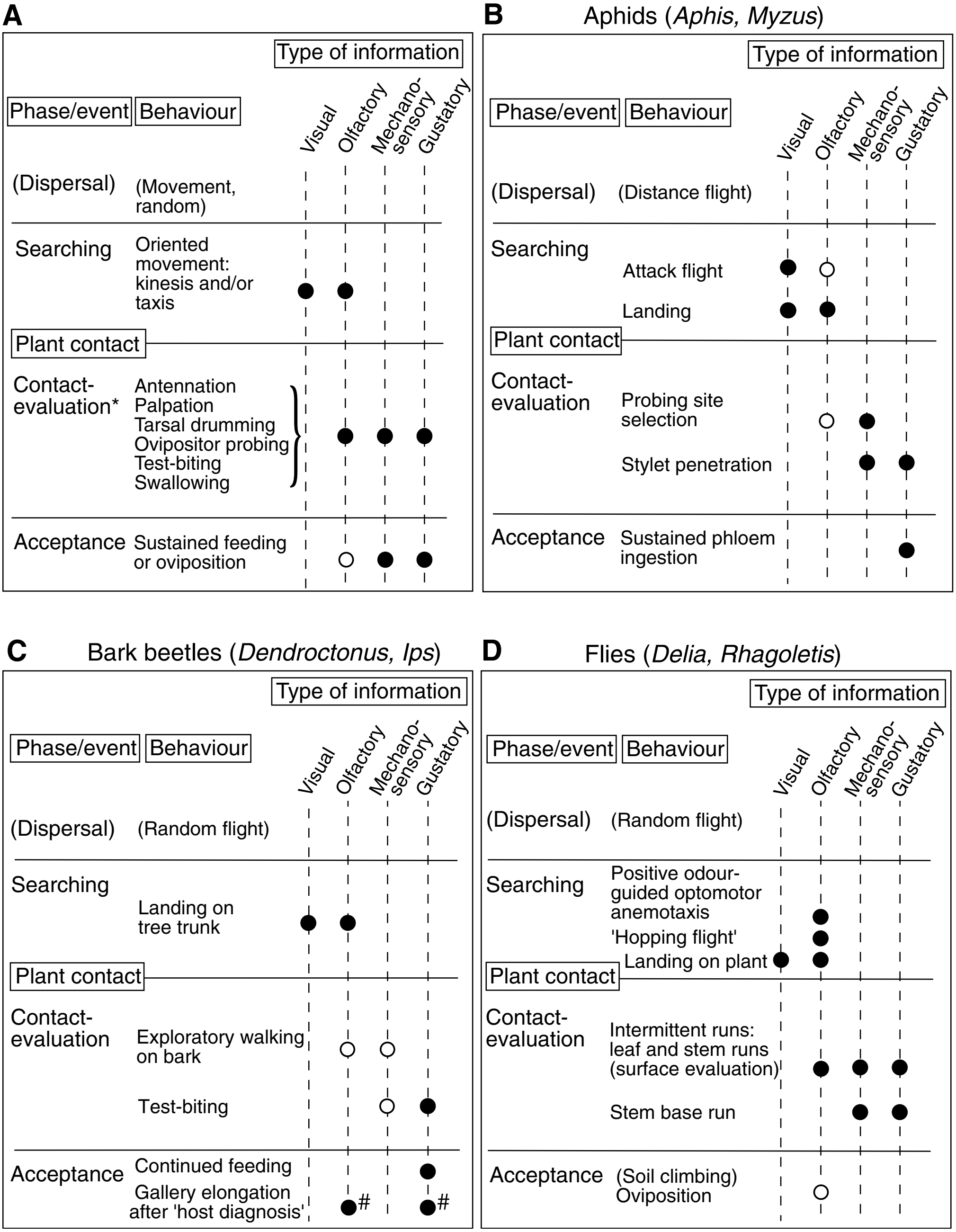


Figure 6.2 (**A**) Generalized sequence of host-plant selection behaviour of herbivorous insects. *Left column*: behavioural phase or event. *Middle column*: common behavioural elements occurring within a behavourial phase. *Right column*: main plant-derived stimuli affecting the behaviour. Black dot indicates well documented plant cue for several species; white dot indicates suggested or probable; asterisk (*) denotes examples of behavioural elements displayed by many species; not all elements occur in a particular species and not necessarily in this sequence. In parentheses at the top, dispersal is indicated as a preceding behavioural phase with its behavioural elements (which do not belong to the host selection sequence). (**B**–**E**) Host selection behaviour sequences of representatives of the four major herbivorous orders, following the scheme of (**A**), with specific elements and terms. (**B**) Alate aphids (*Myzus*, *Aphis* spp.). (**C**) Adult bark beetles (*Dendroctonus*, *Ips* spp.). #Progressive colonization by gallery elongation occurs when repellents or deterrents are absent. (**D**) Adult herbivorous flies (*Delia, Rhagoletis* spp.). For optomotor anemotaxis, visual cues are ground pattern movements, mechanosensory cues are air streams; both not plant-derived. (**E**) Adult nocturnal moths (*Helicoverpa* spp., *Manduca* sexta); optomotor anemotaxis, as (**D**). (Compiled from various sources.)

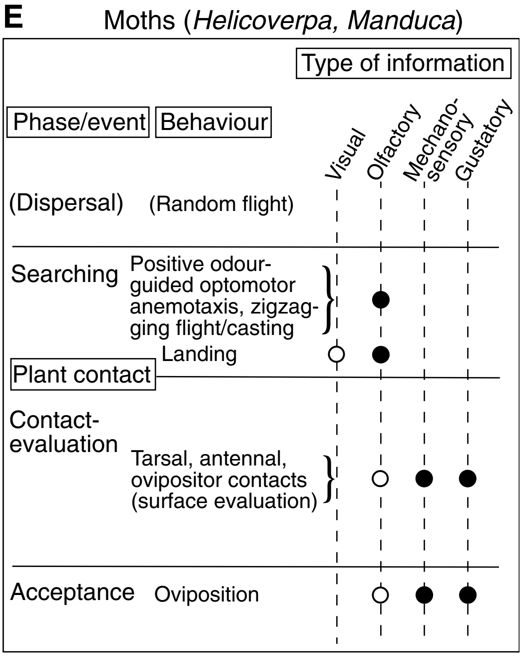


Figure 6.2 (*Continued*)

In these cases, the frequency, rate, and direction of movement appear unrelated to the acceptability of plants within their perceptual range, that is, the range in which host plant-derived cues are detectable by the sensory system. The generation of random movements can be explained by the functioning of so-called 'central motor programmes' located in the CNS. When an insect becomes motivated to search for food, for example because blood trehalose levels fall below a certain level (an internal-state parameter), these programmes are activated and as a result the insect may start a random walk. Only internally stored (e.g. in memory) and proprioceptive information is used.[179] This searching type may be the best possible, either when environmental cues provide no directionality or when the sensory capacity of the insect is insufficient to obtain the required stimuli. During searching, scanning movements may be performed that serve to increase the probability that a resource is detected along the path, mainly because the path is widened. This is seen in caterpillars moving on the ground in search of host plants. The caterpillars raise their heads and first thoracic segments, and sway these from one side to the other.

During random searching, several types of orientation response may be performed upon stimulation by plant-derived cues. These responses may be either non-directed or directed. The non-directional changes in random movement are classified as *kineses*.[89,145] The insect may change its linear speed of movement (orthokinesis) or it may change the rate or frequency of turning (klinokinesis). The intensity of the external stimulus (light intensity, plant odours, humidity, etc.) and the spatial or temporal differences in it determine the strength of these responses. One (unilateral) receptor is sufficient to sense the stimulus intensity by temporal comparisons of incoming sensory information by the CNS. These kinetic responses often lead to area-restricted search (an intensified search in a small area) and arrestment. They are most prominent close to a host plant or upon contact (Figs. 6.3 and 6.4), when the rate of linear movement often decreases and turning rates increase.[107]

Directed movement becomes possible when the host plant emits signals that, either alone or in combination with a second cue, allow directionality to be perceived by the sensory system of the searching insect. Movements in this case are directed by sensory information on external cues but may still be under the influence of central motor programmes (see below). When a distinct directionality towards the food plant results from the analysis of movement patterns, such oriented movements relative to an external source of stimulation are termed *taxes*, and may be towards the source (positive) or away from the source (negative). Orientation to visual or chemical cues, or to their combination, is common to many insects. Over short distances, within a few centimetres, in relatively undisturbed, still air, insects may respond to plant odour gradients by positive chemotaxis. This may be achieved either by temporal comparisons of information coming from the olfactory receptors (klinotaxis) or by comparing sensory input coming simultaneously from a bilateral pair of (olfactory) receptors and trying

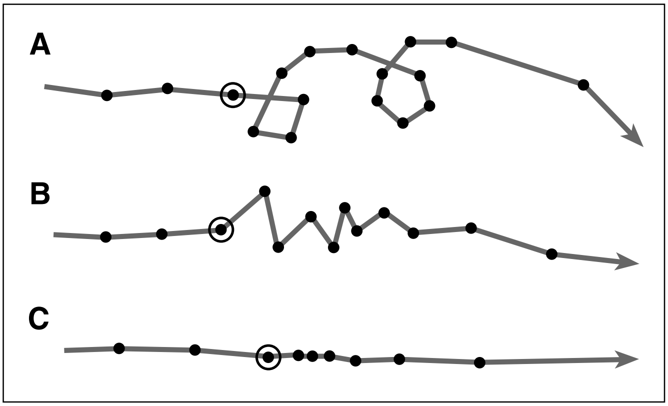


Figure 6.3 Searching patterns used where resources are aggregated. In these cases it may be advantageous for an insect to search an area more thoroughly once it has already encountered a host plant. This strategy increases its chance of finding another host plant. Mechanisms used for restricting the area of search include: (**A**) periodic increases in turning tendency, generating looping or circling; (**B**) alternation in turning direction, generating zigzags; (**C**) adjustments in lengths of moves between stops. Dots indicate landings; circled dots represent landings on host plants followed by egg-laying. (From Bell, 1991.)[17]

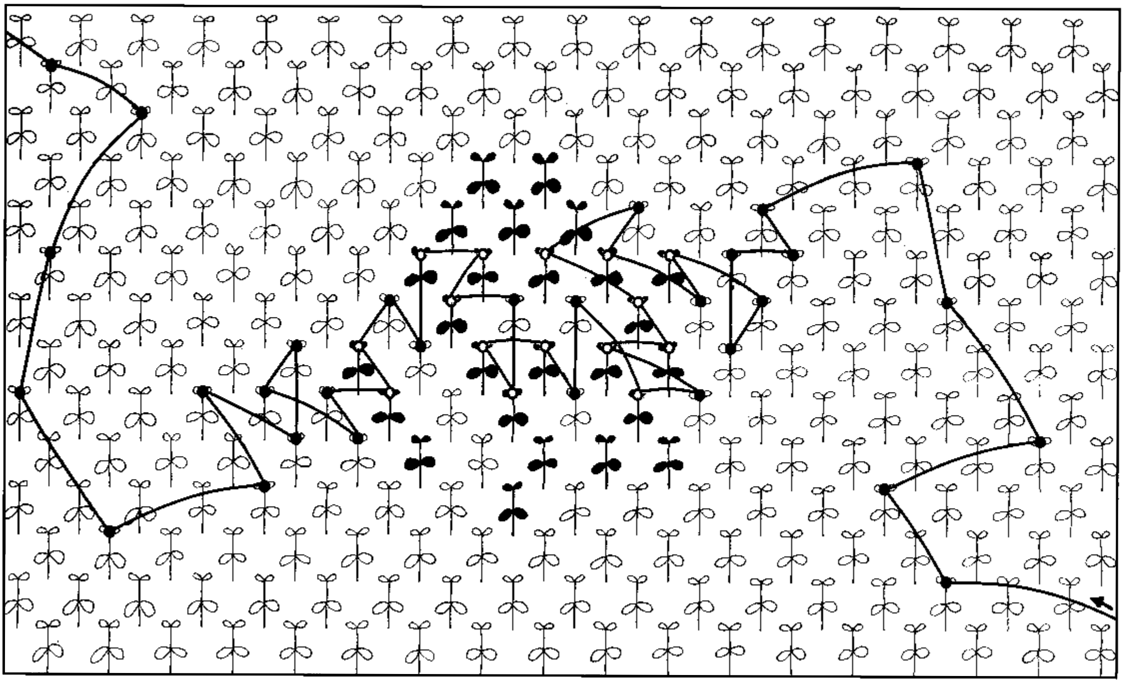


Figure 6.4 Schematized search behaviour in egg-laying females of *Cidaria albulata*, a specialist herbivore on *Rhinanthus* spp. The moths fly shorter distances between alightings and show more turning flight near a host-plant stand, thereby increasing the chance of alighting on a host plant. Turning of flight path and alighting (at least the latter) are stimulated by host-plant odour. Total number of plants, 252; no. of *Rhinanthus* plants, 25 (10%). Total no. of alightings, 45; number of alightings on *Rhinanthus*, 15 (33%). (From Douwes, 1968.)[55]

to obtain equal stimulation of both sides (tropotaxis; symmetrical orientation). A third type of orientation is menotaxis, the maintenance of a constant angle with stimulus direction by preserving a non-symmetrical distribution of sensory stimulation.

Two special cases of menotaxis, *anemotaxis* and *photomenotaxis*, need special attention because they have been found to operate in herbivorous insects. Anemotaxis and photomenotaxis mean oriented movement by maintaining a set angle to the prevailing wind direction or light direction, respectively. Wind or light direction, perceived as air flow by mechanoreceptors or as photon flow by photoreceptors, may be sampled successively at the left and right sides of the body by serial counterturning movements. Wind direction is detected mechanically by walking insects but mainly visually in the case of flying insects. Anemotactic behaviour, influenced by plant odours, is seen in a number of herbivorous insects under laboratory conditions. In contrast to what might be expected, odorous cues do not exhibit a gradient, required for chemotaxis, at distances greater than a few centimetres (see Section 6.4.4). The movement of air in the outside world is mostly turbulent.[111] Odour trails comprise complex plumes actually consisting of discontinuous packets of odour molecules that are moving downwind in random direction. A concentration gradient is absent (Fig. 6.5). The best way

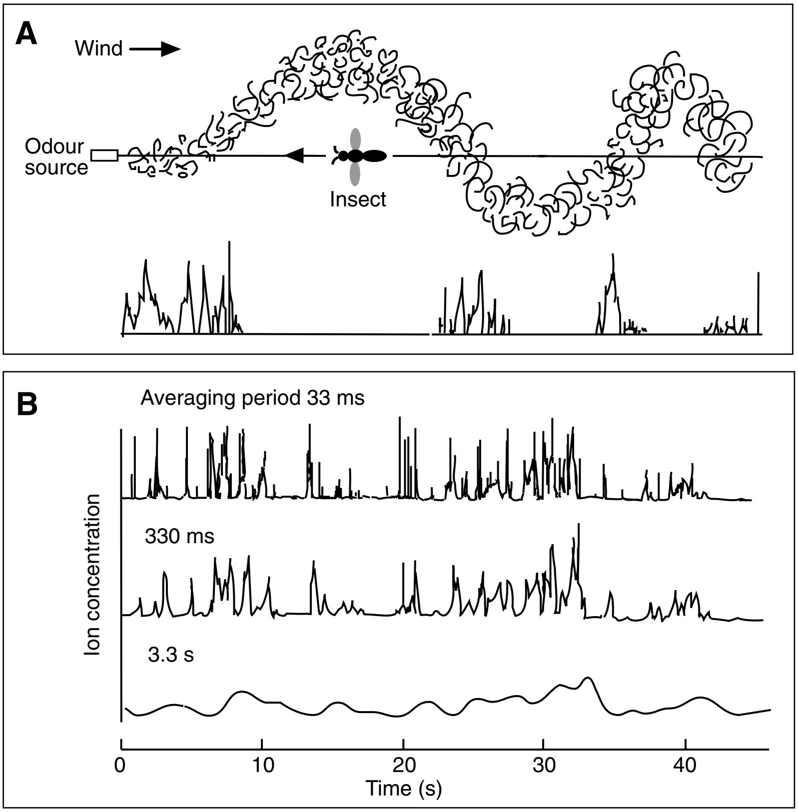


Figure 6.5 (**A**) Schematic drawing of an undulating and meandering odour plume and an odour signal encountered over time when an insect moves upwind in a straight line to a small odour source. (**B**) Signal amplitudes generated by a stationary ion probe located in an odour plume when different averaging periods are used. Packets of odour, resulting from air turbulence, pass the odour receiver. Upon increasing the averaging period, differences in signal amplitude decrease, leading to a decreased resolution of concentration differences by olfactory receptors. However, even at an average time of 3.3 s, the signal is still intermittent and the major bursts of the original can clearly be distinguished. (From Murlis, 1986.)[109]

to minimize the mean time to discovery of the odour source is to explore actively the area surrounding the packet, by performing transverse movements perpendicular to the mean air flow, to increase the rate at which packets of odour are encountered, and locating the midline of the plume. The odour packets are most likely to originate from a cone-shaped space with the top of the cone pointing upwind. The cone-shaped volume is best explored using a zigzag motion until another packet is encountered. This search strategy utilizes simple behavioural rules for movement, combined with knowledge of mean air speed and direction.[11] The resulting movement patterns, casting and zigzagging, match the predictions from theory remarkably well (see Section 6.4.4).

Photomenotaxis, or light compass orientation, is a main mechanism for insects walking on the ground.[145] Although it is difficult to demonstrate anemotaxis in the field, because of lack of control over wind direction and the ubiquitous occurrence of air turbulence, which prevents a consistent directionality and is prominent especially in the boundary layer over the soil surface, the use of photomenotaxis can be investigated relatively simply. One method is Santschi's 'mirror test',[145] and a second method to demonstrate photomenotaxis is the 'turntable test'.[83]

Although the descriptions of movement types and the way in which plant-derived cues may be used are useful to demonstrate the existence of different searching strategies, the number of documented cases for which the orientation mechanism has been fully analysed is small. Especially under field conditions, combinations of mechanisms, rather than a single one, operate under natural circumstances (see Section 6.6). Alternative or additional classifications of searching patterns can be found in the literature.[16,177,179] Models of searching behaviour indicate that, contrary to what one might expect, random walking can be a very effective search strategy and that the rate of random movement is an important factor in determining the success of non-random search.[107] Directed orientation is often viewed as adaptive, as it improves the efficiency of search, that is, it produces a higher success ratio per unit of time and energy invested in searching behaviour.

6.4 Orientation to host plants

6.4.1 Optical versus chemical cues

Two important types of stimuli that could be used as directionality cues by herbivorous insects are optical and odorous characteristics of plants. The relative importance of the two varies between species, as becomes particularly noticeable when diurnal and nocturnal species are compared. The two types of stimulus are often used in an integrated way (see Section 6.6).

The nature of optical and chemical plant-derived cues differs in some important aspects. Light can be characterized by its intensity, spectral composition, and polarization. The unit of light energy, the photon, moves self-propelled at the speed of light. The spectral reflectance pattern of a plant is not substantially altered by air movements and is relatively constant at varying distances from the plant. In contrast, volatile compounds emanating from plants move slowly. In still air they move by diffusion and in all dimensions, but in moving air their concentration in space is highly variable (see below). Odour concentrations rise sharply when the plant is approached. Absolutely still air and complete absence of turbulence are very rare, if not completely lacking, under natural circumstances, and wind speeds are mostly greater than the linear speed of diffusion of organic molecules. In moving air (the normal situation), volatiles are carried away from the source with the prevailing direction of air flow and will be dispersed downwind as packets of odour (see Section 6.3).

In the literature the concept of an odour-filled space has been used that, based on Sutton's model of diffusion, has a semi-ellipsoidal shape in moving air. More recently, however, by the use of ion detectors with a short response time, it has become clear that the odour occurs in a stochastic fashion as packets or filaments of molecules in a meandering plume (Fig. 6.5). Outside the plume boundary, which can be visualized by the use of smoke, no odour packets occur. When moving upwind, the insect may contact spatially separated packets of odour molecules at concentrations only slightly lower than those found close to the plant. Most information on the spatial distribution of odorous molecules comes from studies on the distribution patterns of sex pheromones, which are released from the insect body, virtually a point source. Chapman has stressed the fact that point sources produce odour plumes different from those emanating from big plants or plant patches; clearly, the form of the food source may shape the plume.[33]

In summary, when considering abiotic factors, optical plant characteristics are relatively constant with respect to their distribution and largely independent of temperature and wind speed, but of course they depend on light intensity. Odours emanating from plants have a spatially highly variable distribution and concentration, which depends on wind speed, temperature, and to some extent on light intensity. Moreover, the quality and quantity of emitted plant volatiles may vary depending on the plant's physiological state and on whether it is under attack by herbivores (see Fig. 4.7).[21,160]

Apart from these abiotic factors, the main issues to be considered regarding the relative usability of optical and odorous cues are their specificity and their 'active space', 'effective zone', or 'effective attraction radius'.[26]

Quite often it has been assumed implicitly that optical cues cannot be used to recognize host plants, for the reason that 'all plants are green' (i.e. the dominant reflectance–transmittance hue is 500–580 nm). In apparent contrast, several plant species have been found to emit volatile chemicals or chemical blends that appear to be taxon specific, either qualitatively (unique compounds) or quantitatively (characteristic ratios).[177] This has probably led to the greater attention paid in the literature to odours as guiding factors in host-plant searching, especially in the case of specialized herbivores. In contrast to the low variability of spectral composition of light reflected by foliage, however, intensity of reflected light may differ more pronouncedly between species, because of the presence of wax crystals or trichomes on the leaf surface, or because of biotic (age, nutrient status) and abiotic (density, incident light intensity, background) factors.

The maximum distance over which plant cues can guide an insect to its host plant is another important factor related to the concept of *active space*. Active space is defined as the space within which the intensity of a stimulus or cue is above the threshold for a behavioural response. In the absence of visual cues, behavioural responses to plant odours have been demonstrated at distances of 5–30 m for several oligophagous species, with a maximum of 100 m reported for the onion fly *Delia antiqua* (Table 6.2). The fact that some insects can be lured to scented traps suggests that volatile plant compounds may under field conditions attract herbivorous insects, sometimes over large distances. Tephritid fruit flies and diabroticite rootworm beetles can be attracted in large numbers to traps baited with specific blossom aroma components. This applies also to some polyphagous species, such as corn earworms[77] and Japanese beetles. The latter may be attracted in open areas to such traps from a distance of up to 400 m. In these cases, volatile-baited traps appear to be an effective and sensitive tool for monitoring insect densities.[102]

The significance of values on linear distances and conclusions about active spaces under natural conditions depend heavily on both the biomass and the complexity of the vegetation, factors that have not been varied extensively in field studies on insect host-plant searching. The integrity (unmixed character) of the stimulus produced by an individual host plant or a patch of host plants in a mixed plant stand is thought to be preserved over relatively short distances only,[162] although in some instances odours may remain attractive despite

Table 6.2 Distances over which odorous or optical plant cues have been shown to elicit positive taxis-type responses from herbivorous insect species

Insect species	Distance (m)	Reference
Odorous cues		
Leptinotarsa decemlineata	0.6	83
	6	49
Ceutorhynchus assimilis	20	58
Delia radicum	24	63
Dendroctonus spp.	30	181
Pegomya betae	50	138
Delia antiqua	100	85
Optical cues		
Delia brassicae	2	128
Empoasca devastans	3.6	142
Leptinotarsa decemlineata	8	171
Rhagoletis pomonella	10	3

mixing with other plant volatiles. Thus, gravid beet flies (*Pegomya betae*) are attracted by the odour of young beet leaves over distances of up to 50 m, even if these odours have passed non-host plants.[138] Optical contrasts in a mixed plant stand may be perceived over distances of a few metres, especially in flying insects. At present, few firm data exist on the size of active spaces based on either optical or odorous signals, and the conclusion that the active space of odorous signals is greater than that of optical cues[19,128] seems premature. Indeed, under field conditions they always occur together and it will be shown below (see Section 6.6) that insects use combinations of signals, which may enable them to overcome the disadvantage inherent in relying solely on either one.

6.4.2 Visual responses to host-plant characteristics

Three optical characteristics of plants may influence host selection behaviour: spectral quality, dimensions (size), and pattern (shape).[128] The spectral sensitivity of insect compound eyes ranges from 350 to 650 nm (near-ultraviolet to red) and thus includes shorter wavelengths than that of the human eye (Fig. 6.6). The ommatidium, the basic photoreceptor and image-formation unit of the insect compound eye, is of a fixed-focus type. This results in maximum acuity at very close range, whereas at greater distances perception of shape is poor. For a more detailed discussion of characteristics of photoreceptors and the sophisticated visual system of insects, the reader is referred to other texts.[24,153] Although the size of plants or plant parts and their shapes show considerable variation between and within plant species, this variation presumably aids plant selection only at close distances.

To illustrate the extent to which visual discrimination is used in host-plant selection, examples of insect responses to optical host-plant cues, such as shape and colour will be presented.

(a) Lepidoptera

The responsiveness of day-foraging butterflies to colours has been relatively well studied. When

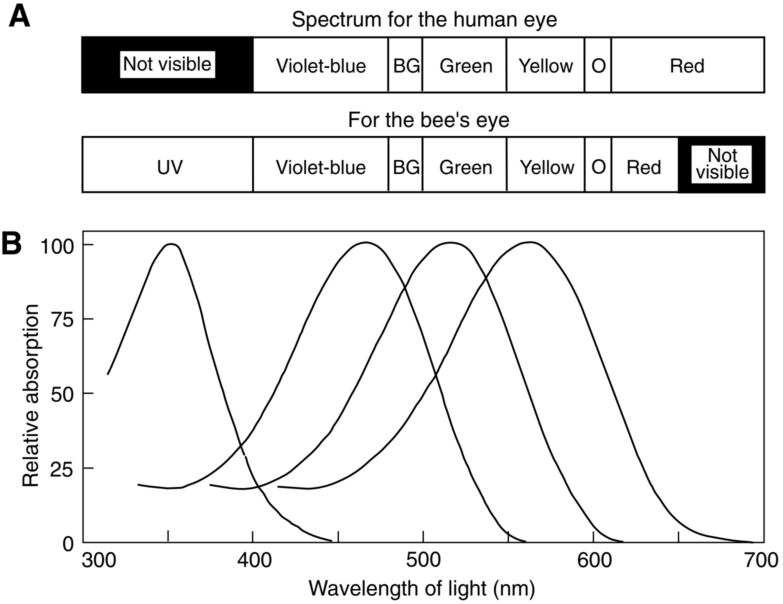


Figure 6.6 (**A**) Comparison of the wavelength spectra (nm) perceived by humans and honeybees. (Data from Chittka and Waser 1997.)[35] (**B**) Spectral sensitivity curves of a tetrachromatic insect eye (*Spodoptera* sp.). The absorption of each pigment is expressed as a percentage of the maximum for that pigment. (From Langer *et al.*, 1979.)[97]

artificial leaves of green paper are offered to gravid cabbage white butterflies (*Pieris brassicae* and *P. rapae*), naive individuals show landing responses, albeit at much lower frequencies than in response to cabbage leaves. Immediately upon alighting on the substrate they start to 'drum' it for a few seconds, even though specific host-plant chemicals are absent. For *P. brassicae*, true colour vision and wavelength-specific behaviour have been demonstrated (Fig. 6.7), and *P. rapae* clearly showed landing preferences for differently coloured artificial substrates. In both *P. brassicae* and *P. rapae*, associative learning (see Chapter 8) in response to different shades of green has been demonstrated.[169,174] These butterflies switch their colour preference for landing responses from the green colour of leaves to the yellow, blue, and violet colours of flowers, depending on their motivation for oviposition or nectar feeding, respectively. In the papilionid butterfly *Battus philenor*, discrimination of leaf shape has been demonstrated, and this butterfly uses leaf shape as an associatively learned signal for preferential landing on host plants.[121] The pierid butterfly *Eurema hecabe* prefers artificial leaves having longer contours, corresponding with the compound leaf shapes of its fabaceous hosts.[79] The butterfly *Papilio aegeus*, a specialist of rutaceous plants, responds to the false colours that arise from polarization of reflected light. Its photoreceptors combine sensitivity to colour with sensitivity to polarization, whereas in other insects such as the honeybee these occur in separate ommatidia. Leaf surface traits, such as glossy or glaucous appearance, affect the polarization of reflected light, as do variations in the vertical/horizontal plane of leaf orientation. Perception of false leaf colours might thus guide oviposition site selection.[88] The nocturnal moth *Mamestra brassicae* prefers to land on medium-sized yellow-coloured artificial substrates that are offered in a vertical position. The combination of an optical target with host-plant odour increases landing probability.[135]

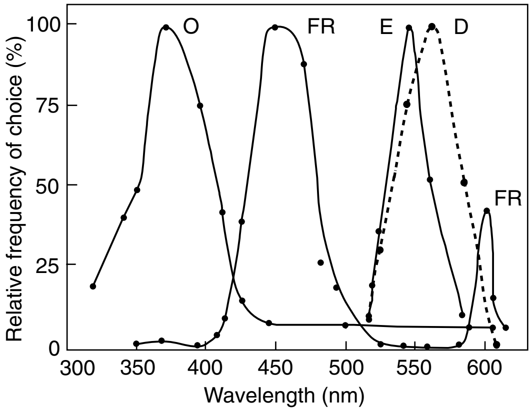


Figure 6.7 Relative effectiveness of different wavelengths in eliciting behavioural responses from *Pieris brassicae* butterflies. The *y*-axis gives the relative frequency of choice (%), normalized to the maximally visited wavelength (=100%). A so-called open-space reaction (O, lack of response to plants and tendency to increase flight altitude) is induced by wavelengths in the ultraviolet range; a feeding reaction (FR, extension of the proboscis) is maximally induced by blue and to a lesser extent by yellow, and egg-laying (E) and drumming (D) by slightly different wavelengths in the green part of the spectrum. (From Scherer and Kolb, 1987.)[143]

Despite the fact that single rhabdome stemmata of caterpillars are very simple organs compared with the compound eye of the adult butterfly, caterpillars are able to discriminate object sizes and colours, enabling them to orient towards plant silhouettes after dropping to the ground.[101,132,142]

(b) Diptera

In the case of herbivorous flies among the families Tephritidae (fruit flies) and Anthomyiidae (root maggots), the use of visual cues has been amply demonstrated.[127] For a flying *Rhagoletis pomonella* female in search of oviposition sites (i.e. apple fruits), the sequence of visually oriented behaviour can be described as a series of consecutive steps. At a distance of about 10 m, a single tree is perceived as a silhouette contrasting against the background. Perception of colour is unlikely at this stage, especially when the insect is facing direct sunlight, as is the perception of details of shape, because of its limited visual acuity. When the fly is at a distance of a few metres or less from the plant and finds itself either in front, under, or above the tree crown, spectral quality and intensity of the reflected light are the main cues evoking alightment on, for instance, foliage, fruits, or trunk. At still closer range (1 m or less), as a third step, detailed

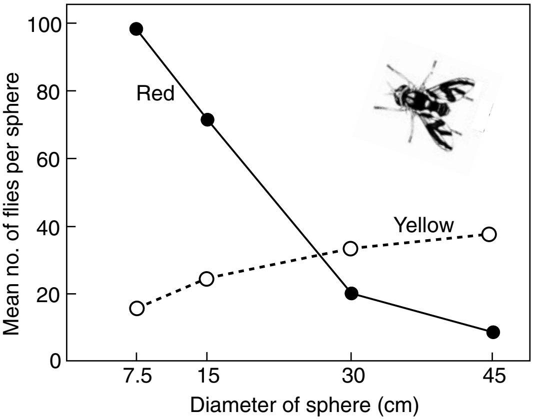


Figure 6.8 Visual responses of apple maggot flies (*Rhagoletis pomonella*) to red and yellow odourless sticky spheres of increasing diameter under orchard conditions. Visual preferences were measured on the basis of catches of flies on the spheres. A red sphere of 7.5 cm in diameter matches the size and colour of a ripe apple. The higher number of flies caught on larger yellow spheres is interpreted as a response to a supernormal substitute stimulus for the green of leaves, on which the flies search for aphid honeydew as a source of energy. (From Prokopy, 1968.)[126]

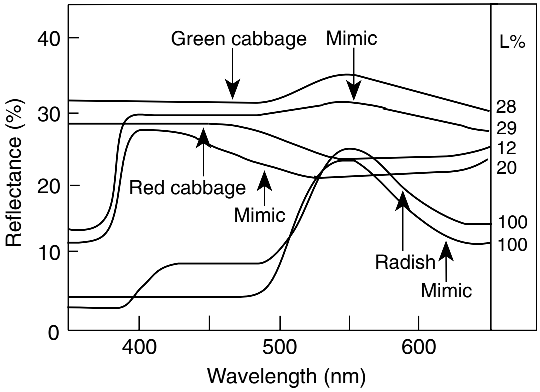


Figure 6.9 Reflectance properties of leaves of different cruciferous host plants and landing responses of cabbage root flies (*Delia radicum*) to real leaves or artificial mimics of these. Landing responses (L%) are expressed as the percentage of landings relative to radish, the plant on which the flies landed most frequently in a multiple choice test. Alternative host plants were green cabbage and red cabbage. In direct comparisons of real leaves and their mimics, flies landed with equal frequencies on both. Vertical axis gives the percentage of reflectance of incident light. (Redrawn from Prokopy *et al.*, 1983a.)[129]

discrimination on the basis of size or shape becomes possible (Fig. 6.8).

In the cabbage root fly *Delia radicum*, visually based landing responses occur when the flies are offered artificial leaves that have been painted with colours mimicking host-plant leaf reflectance profiles (Fig. 6.9). When spectrally matched artificial leaves of three different host plants were offered simultaneously with the real leaves, no landing preferences were found. The flies shifted their preferences with plant age. The overriding preference for radish in the mature plant stage was much less pronounced in the young plant stage and this correlated with smaller differences in reflectance properties between the three host plants. During the post-alightment phases of host selection, leaf shape does not seem to influence oviposition, but artificial leaves possessing a stem are clearly preferred over those lacking one (Fig. 6.10).

When the flies were allowed to choose between different sizes of artificial leaf, the one that was four times as big was also landed on four times as often and received 2.5 times as many eggs.[130,133] Colour preferences of a polyphagous and an oligophagous species of tephritid *Bactrocera* fruitflies were clearly different. The polyphagous species *B. tryoni* preferred blue artificial spheres reflecting ultraviolet light (UV) over spheres lacking this reflectance.[56] This UV sensitivity is functional, as ripe natural host fruits have heavier waxblooms causing stronger UV reflection. Clearly plant colour, shape, and size play important roles in the host selection behaviour of these herbivorous flies, which belong to the best studied species in this respect. Visually guided behaviour is also influenced by odour perception (see Section 6.6).

(c) Homoptera

Attraction to the colour of foliage has been studied extensively in aphids and whiteflies.[37,91,106] These small insects can generate only small motoric forces, and at wind speeds exceeding 1 m/s they are unable to maintain their airspeed against the wind direction. They are able, however, to exert active control over their groundspeed.[81] Alate (i.e. the winged morph) aphids can still exert control over their transport by active taking off and alighting. The main factor that elicits an alighting response is the perception of plant colours. Thus *Brevicoryne brassicae* and *Myzus persicae* alight in the field

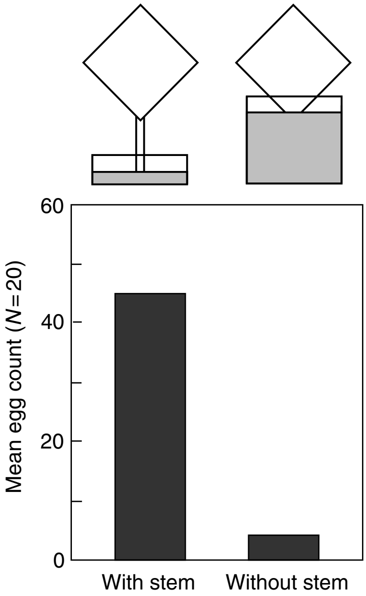


Figure 6.10 Effect of the presence of a stem as a morphological feature of artificial leaves on oviposition preference of cabbage root flies (*Delia radicum*). Artificial leaves (13 × 13-cm pieces of green paper dipped in paraffin and sprayed with a surface extract of cabbage leaves) of each type were offered together in the same test arena. (From Roessingh and Städler, 1990.)[133]

preferentially on leaves reflecting a greater proportion of long-wave energy, with little or no regard for the taxonomic status of the plants. As sugar beet leaves have a higher 'long/short-wave ratio' than cabbage leaves (Fig. 6.11), more cabbage aphids alight on sugar beet leaves than on cabbage, although the former is not one of its hosts. 'Long/short-reflectance ratios' change with leaf age and water status. The colour attraction of these 'yellow-sensitive' aphid species serves to bias their landings towards plants of the appropriate physiological type rather than to recognize their host-plant species.[91]

Likewise, *Aphis fabae*, which alights three times as frequently on beet *Beta vulgaris* plants as on reed (*Phragmites communis*), has a preference for saturated yellow, which more closely resembles the reflectance profile of *Beta* leaves (Fig. 6.12). The mealy plum aphid *Hyalopterus pruni* displays so-called host alternation (see Section 8.4.1) between its summer host *Phragmites* and its winter host *Prunus* spp. Alates, which search for *Phragmites* in the spring, alight twice as often on reed plants than on adjacent non-host beet plants.[106] Discrimination between these two plant species is done in this case on the basis of a lower degree of saturation of the yellow reflectance of the *Phragmites* blades compared with that of *Beta* leaves. Thus, the visually based response to colours and reflectance intensity is species specific. Whiteflies avoid settling in the presence of short-wavelength illumination (400 nm), but will alight on green light (550 nm).[37] As with butterflies and flies, plant-surface wax loads may also affect visually guided host-plant selection behaviour in aphids. Early in the season, alates of the pea aphid *Acyrthosiphon pisum* are found at lower density on an isoline of pea *Pisum sativum* with reduced surface wax than on peas with a standard surface wax bloom.[184]

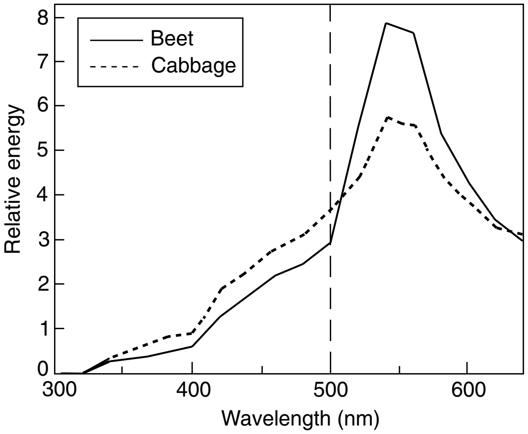


Figure 6.11 Relative energy curves of light reflected from the upper surfaces of mature leaves of sugar beet and cabbage in July under direct sunlight. The ratio between the areas under the curve to the right and left of the dashed line at 500 nm (the 'long/short ratio') is 3.2 for beet and 2.1 for cabbage. (From Kennedy *et al.*, 1961.)[91]

Not only lepidopteran, dipteran, and homopteran insects, but also species belonging to other orders, use differences in reflectance intensity between plant species, or between leaves or organs within a plant, as a visual selection criterion for more nutritious tissues. These are often younger tissues, which display a relatively strong reflection in the yellow region. In fact, most diurnal insects are attracted to yellow. In many cases yellow surfaces act as a 'supernormal' stimulus, because

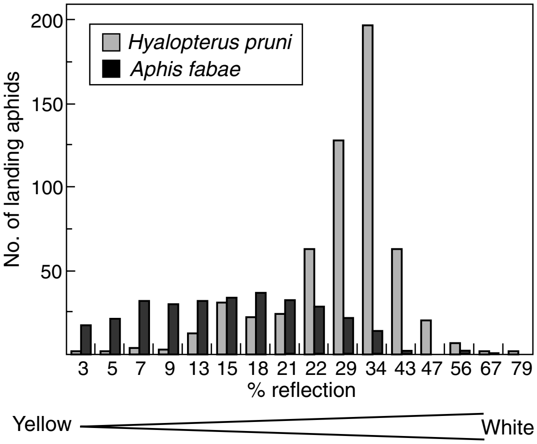


Figure 6.12 Visual landing preferences of two aphid species (*Hyalopterus pruni*, the mealy plum aphid, and *Aphis fabae*, the black bean aphid). Preference was measured as the number of alate aphids that landed on each of 16 plates, which together constituted a graded series from yellow to white colours with increasing reflection in the short wavelength band (decreasing saturation from left to right). (From Moericke, 1969.)[106]

they emit peak energy in the same bandwidth as foliage, but at greater intensity.

Although there is a large body of information on the mechanisms of insect photoreception, our knowledge of the visual performance of herbivorous species in the field is limited relative to what is known about olfactory performance, discussed in the following sections.

6.4.3 Olfactory responses to host plants

When attempting to test the separate role of visual stimuli, test insects are exposed to objects with controlled optical characteristics, which are odourless. Conversely, to assess the effect of odours alone on orientation to host plants, the visual surroundings in which the odour tests are carried out should be homogeneous. For experiments in the laboratory, several set-ups have been developed that allow quantitative studies of orientation responses to odours (see Appendix C2).[61] As discussed above (see Section 6.4.1), control over an odorous stimulus in terms of concentration and distribution is usually less exact than is often assumed. We will discuss in more detail two examples of orientation mechanisms to odours as demonstrated under laboratory circumstances, one of a flying insect and one of a walking insect.

6.4.4 Flying moths and walking beetles: two cases of olfactory orientation

When a flying female tobacco hornworm moth (*Manduca sexta*) is searching for a host plant, she displays positive anemotaxis, that is, she flies upwind using the prevailing direction of air flow as a cue. Mechanoreceptors located on her antennae and serving as anemoreceptors provide this directional information (either by klinotaxis or tropotaxis; see Section 6.3). Her flight path can be described as a regular zigzag (a series of counterturns) of limited amplitude.

How does the odour emitted by the tobacco plant come into play? First, the host-plant odour may have acted as an activator (arousing agent) for flight to occur, by inducing the moth to take off from a resting or walking condition. Once in flight, she may pick up an odour plume emanating from one or a group of host plants, and her subsequent flight path is then determined mainly by trying to prevent loss of the odour plume. When, over a certain minimum time interval, olfactory receptor cells do not detect odour, a so-called 'casting' response ensues. The moth reduces speed and increases the amplitude of the counterturns, thereby flying more across wind and regressing in a downwind direction. When, during casting, odour molecules are picked up again by the olfactory sensilla, upwind zigzagging is resumed. This sequence of behavioural acts may be reiterated until final approach of the host plant. Closer to the odour source the intervals between counterturns decrease. This host-searching mechanism is designated as odour-conditioned (or odour-modulated) positive anemotaxis.

The female's host-plant searching behaviour is in fact very similar to the odour-modulated upwind flight of male moths in search of a female.[10] In the latter case the odorous signal is a sex pheromone emitted by the female. A present view of the mechanisms steering this behaviour maintains that the serial counterturning is controlled by a motor programme in the CNS that is set in motion by olfactory activity, but afterwards is continued automatically (self-steered).[187] The switch from zigzagging to casting, however, is controlled by olfactory information: absence of activity changes in the odour receptors over a certain minimum timespan causes casting behaviour. Upwind progress is made possible by optomotor feedback, that is, the flow of visual images of the surroundings, mainly the ground, controls the motor response via a feedback loop.

The female is able to maintain the parameters of its flight path (ground speed, track angle) and counterturning frequency close to some apparently preferred values over a range of wind speeds. Odour-conditioned anemotactic flight enables directed flight to an odour source and is basically different from the relatively straightforward chemotactic orientation to odour gradients. It has probably evolved because, as we have seen, such gradients do not exist over any distance in the field. Behavioural mechanisms employed in sex pheromone-guided mate-finding in male insects have been relatively well studied,[59] including temporal and spatial aspects.[94] However, information is still scanty for orientation mechanisms to plant odours under field circumstances.[100,187] One of the best studied cases of the ability of a walking insect to orient to host-plant odours is the Colorado potato beetle *Leptinotarsa decemlineata*.[177] This specialist on solanaceous plants has a strong preference for the cultivated potato *Solanum tuberosum*, on which it is one of the most devastating insect pests. During the first 7 days of adult life the beetles need to feed in order to develop their flight muscles fully and, as a consequence, host-plant location is done by walking. To quantify their walking behaviour, a 'locomotion compensator' in combination with a wind tunnel has been used. This instrument allows detailed and automated recording of walking tracks without the insect contacting any obstacles (see Appendix C2).

When clean air is blown over a hungry beetle, it shows a menotactic response to the wind (anemotaxis), maintaining a relatively constant angle to the wind direction (Fig. 6.13). The walking track shows circling by making turns of 360°. When the airstream carries the odour of intact potato plants, the straightness of the path increases dramatically. Now that circling is absent, average walking speed is increased and the beetles spend more time walking upwind. This response can be classified as positive (i.e. upwind) odour-conditioned anemotaxis. When the odour of non-hosts, for instance cabbage plants, is offered, the track parameters are similar to those recorded for clean air. When the odour of potato plants is combined with that of cabbage plants, the orientation response to potato is neutralized and the walking tracks of the beetles cannot be distinguished from those performed in clean air (Fig. 6.13).

Somewhat unexpectedly, similar effects were found when the odour of another solanaceous plant, wild tomato (*Lycopersicon hirsutum*

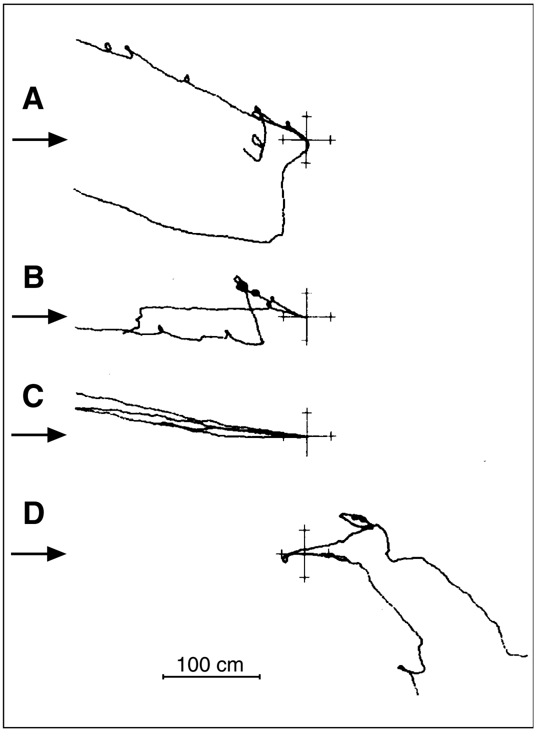


Figure 6.13 Walking tracks of an individual female Colorado potato beetle (*Leptinotarsa decemlineata*) during four consecutive periods (**A**–**D**) of 10 min. The stimulus situations were: (**A**) clean air stream; (**B**) air stream carrying the odour of cabbage (*Brassica oleracea*) plants; (**C**) air stream carrying the odour of potato (*Solanum tuberosum*) plants (the favourite host plant of the beetle); (**D**) air stream carrying a mixture of odours emanating from cabbage and potato. Arrows indicate the direction of the air stream. The plotter reset the position of the beetle to the origin (centre of cross) after a certain maximum distance had been travelled. Total distance travelled and track straightness are significantly higher for (**C**) than for the other three situations, which do not differ from one another. (From Thiéry and Visser, 1986.)[162]

Table 6.3 Selected cases of adult herbivores belonging to four major insect orders that display behavioural responses to plant odours; the insect's host-plant specificity, type of odour source, test environment, and availability of sensory data are indicated

Order and species	Specialization category	Odour source	Type of test environment	Sensory data	Reference
Hemiptera					
Phorodon humuli	M	G	L/F	SCR	28
Cryptomyzus korschelti	O	HP	L		180
Cavariella aegopodii	O	G	F(T)		34
Lipaphis erysimi	O	S	L	SCR	118
Brevicoryne brassicae	O	S	L(F—)	SCR	118, 123
Rhopalosiphum padi	O	G	L(F—)		122
Aphis fabae	P	HP	L(F—)	SCR	90, 118
Aphis gossypii	P	HP	F		125
Coleoptera					
Leptinotarsa decemlineata	O	HP/G	L(F—)	EAG/SCR	98, 162, 176
Anthonomus grandis	O	G	L/F	EAG/SCR	52, 53, 54
Ips typographus	O	G	L/F	SCR	105, 168
Phyllotreta spp.	O	S	L/F		124
Ceutorhynchus assimilis	O	S/HP	L/F	SCR	22, 58
Popillia japonica	P	G	F		1
Listroderes obliquus	P	G/S*	L		99
Oreina cacaliae	O	HP/HPE	L		86
Phyllopertha diversa	P	G	L	SCR	74
Hylobius abietis	O	HPE	L	SCR	186
Diptera					
Psila rosae	M	S	L/F	EAG	70, 71, 117
Delia antiqua	O	S	L/F	EAG/SCR	70, 80, 85
Delia radicum	O	S	L/F	EAG	40, 70, 117
Rhagoletis pomonella	O	G	L/F	EAG	60, 65, 114
Dacus dorsalis	P	G	L/F	EAG	102
Lepidoptera					
Helicoverpa subflexa	M	HPE	L		165
Acrolepiopsis assectella	M	S	L		161
Plutella xylostella	O	HPE	L	EAG	120
Manduca sexta	O	G/HP/HPE	L	EAG	164, 100
Papilio polyxenes	O	G	L	EAG	15
Helicoverpa virescens	P	HPE	L	SCR	82, 137, 166
Trichoplusia ni	P	HP	L		96
Ostrinia nubilalis	P	HP/G	L	EAG	29, 170
Spodoptera littoralis	P	HP/G	L	SCR	84, 141
Mamestra brassicae	P	HP/HPE/S/G	L	EAG	134, 136
Cydia pomonella	O	G/S	L/F	EAG	6, 38, 76

M, monophagous; O, oligophagous; P, polyphagous; HP, intact (host) plants; HPE, host-plant extract; G, generally occurring green-leaf volatiles; S, volatile(s) specific to the host plant taxon; L, behavioural test in the laboratory, in an olfactometer or a wind tunnel; F, field test, either trap catches (F(T)) or direct observations; (F—), behavioural responses to the odour source attractive under laboratory conditions could not be demonstrated under field conditions; EAG, data on sensory perception of volatiles from the odour source available at the electroantennogram level; SCR, data on sensory perception of volatiles from the odour source available at single-cell level.

* The specific volatiles were isothiocyanates, which are characteristic for Cruciferae, one of the preferred host-plant families.

f. *glabratum*), was offered. This is an unsuitable plant for the beetle. Despite the taxonomic relatedness of tomato to potato, mixtures of their volatiles were not attractive to the beetles. The phenomenon that the presence of tomato odour prevents the beetles from orienting to their host plants has been termed 'odour masking'.[163] It has been suggested that this phenomenon plays a role in reducing population levels of herbivorous insects in mixed cropping systems (see Chapter 13).

Positive odour-conditioned optomotor anemotaxis and olfactory-induced visual orientation are presently considered to be the main mechanisms used during host-plant searching in herbivorous insects, in both specialized and polyphagous species.[154,177] In addition, there is evidence that chemotaxis occurs within ranges of a few centimetres from the host plant, as has been demonstrated for several caterpillars and various root-feeding insects.[93,115] Table 6.3 presents a selected summary of data on behavioural responses to plant odours in adults of herbivorous species belonging to four major orders. In each order, food specialists have been found to respond to identified odours specific to their host plant.

Generalist herbivores have been shown to exploit plant volatiles as signals conveying information on plant condition, thereby serving to optimize host-plant selection. The polyphagous *Myzus persicae* is more strongly attracted to and arrested by potato plants that are infected by potato leafroll virus, which have a higher host-plant quality than uninfected plants.[57] However, the generalist moth *Helicoverpa virescens* avoids ovipositing on plants damaged by conspecific caterpillars. Damaged plants emit specific volatiles only during the dark phase and these strongly repel nocturnally active female moths in search of an oviposition site.[42] Not only herbivorous insects, but also many of their arthropod natural enemy species, exploit plant volatiles as infochemicals (see Chapter 10).[51,157]

6.5 Chemosensory basis of host-plant odour detection

Insects rely heavily upon chemoreception when searching for food, oviposition sites, and mating partners, as well as for social communication. In this context it is often stated that 'insects live in a chemical world'. Chemoreception refers to the classical senses of smell (olfaction, organs for detecting volatile chemical stimuli) and taste (gustation, or 'contact chemoreception' for the detection of dissolved or solid chemicals; see Chapter 7). The distinction between the two is not absolute, as insect taste sensilla have occasionally been found to respond also to odours,[156] and members of a gustatory receptor protein family[36] are expressed in the antenna and have olfactory functions.[183]

6.5.1 Morphology of olfactory sensilla

Olfactory chemoreceptor cells are associated with so-called *sensilla* (singular: sensillum), organs consisting of neurons, accessory cells, and a cuticular structure (Fig. 6.14).

The cell bodies (perikarya) of the neurons are closely associated to the externally visible cuticular structure. The dendrites are usually located in specialized cuticular structures, which are classified on the basis of external form. They include hair-like varieties (sensilla trichodea), pegs and cones (sensilla basiconica, often involved in plant odour perception), pegs or cones sunk in shallow depressions (sensilla coeloconica), and pore-plate organs (sensilla placodea). Typically there are two to five neurons in olfactory sensilla,[32,87,112] but in locusts up to 50 neurons may innervate one sensillum basiconicum. In pore-plate sensilla of the honeybee *Apis mellifera* up to 30 neurons innervate one sensillum placodeum.[69] Chemosensory neurons are mostly bipolar and their axons run to the CNS via peripheral nerves without intermittent synapses. The dendrite, a filament-like extension of the neuron that protrudes into the sensillum cavity, is specialized to respond to the chemical stimulus with a graded potential called the receptor potential. When this potential reaches a value above a certain threshold, it gives rise to a train of action potentials.

There are some important structural differences between olfactory and gustatory sensilla. Olfactory sensilla are multiporous, the entire sensillum wall or plate is perforated by up to thousands of minute pores (diameter about 10–50 nm), and dendrites are often branched.[158] In contrast, gustatory sensilla are

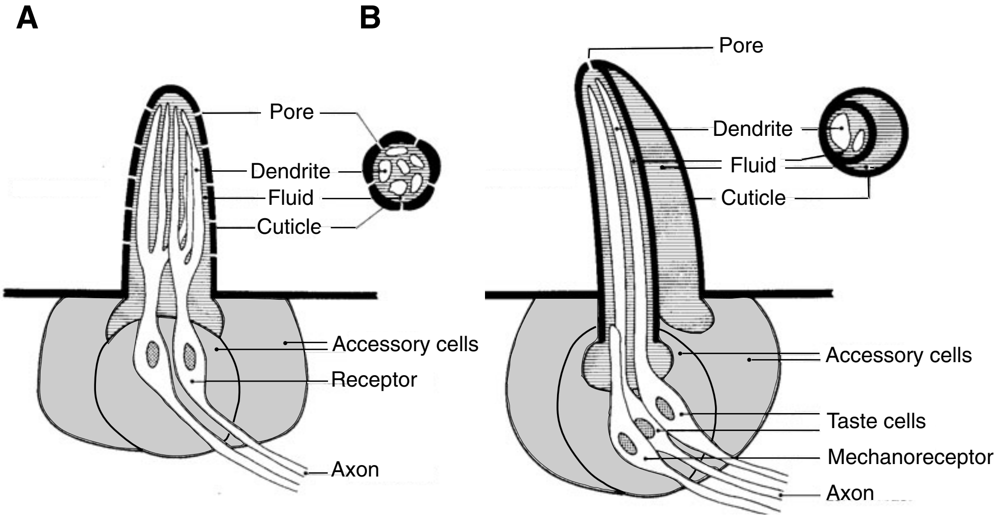


Figure 6.14 Schematic drawing of longitudinal and transverse sections of (**A**) an insect olfactory hair and (**B**) an insect taste hair. The olfactory hair is innervated by two bipolar chemoreceptor neurons; the taste hair is innervated by two chemoreceptors and one mechanoreceptor. (Courtesy of Dr. F.W. Maes, Groningen State University, The Netherlands.)

uniporous, the pore (diameter 200–400 nm) mostly being located at the very tip of a peg-, hair-, or papilla-like sensillum (Fig. 6.14). In both cases the dendritic tips are close to the pores, but are protected from desiccation by receptor lymph, which is secreted into the sensillum lumen by the tormogen and trichogen cells at the sensillum base. Olfactory sensilla are predominantly present on antennae, but may also occur on maxillary and labial palpi and on the ovipositor. The number of olfactory sensilla and the olfactory receptor cells associated with them is quite variable between species. Larvae of holometabolous insects have only small numbers of olfactory cells (e.g. less than 10 neurons for beetle larvae and about 100 for fly maggots and caterpillars[183,188]). For female adults, this number amounts up to a few hundreds in Hemiptera, whereas for Lepidoptera it varies between 6500 and 177 000 (in female *Manduca sexta*) per antenna.[32] The sensilla that house olfactory receptor cells may be multimodal, that is, they may also contain thermo-, hygro-, and mechanoreceptors.[47,147]

6.5.2 Olfactory transduction

The transduction process—the process by which quality and quantity of the chemical stimulus is converted into a receptor potential and eventually into action potentials—involves a sequence of steps. The recent past has brought considerable progress in the understanding of the molecular basis of olfactory transduction. A current model is depicted in Figure 6.15. The initial event is the diffusion of volatile stimulus molecules into the sensillum lumen via the pore(s) in the sensillum wall and binding of these molecules to small (14 kDa) water-soluble odorant-binding proteins (OBPs), which carry the volatile stimulus molecules (ligands) to the olfactory receptor (OR) molecules present in the dendritic membrane. Either the stimulus molecule or the complex of OBP and stimulus molecule then binds to these membrane receptors. ORs in insects are G protein-coupled (GPC) seven-transmembrane proteins acting through activation of second messengers such as cyclic adenosine monophosphate (cAMP) or inositol 1,4,5-triphosphate (IP3) involved in the opening of ion channels in the dendritic membrane. Opening of ion channels leads to depolarization of the dendritic membrane. When the magnitude of the depolarizing receptor potential exceeds a threshold, this results into generation of action potentials that travel over the axonal membrane to the glomeruli in the antennal lobe of the CNS (see below). The activity of stimulus molecules is most probably

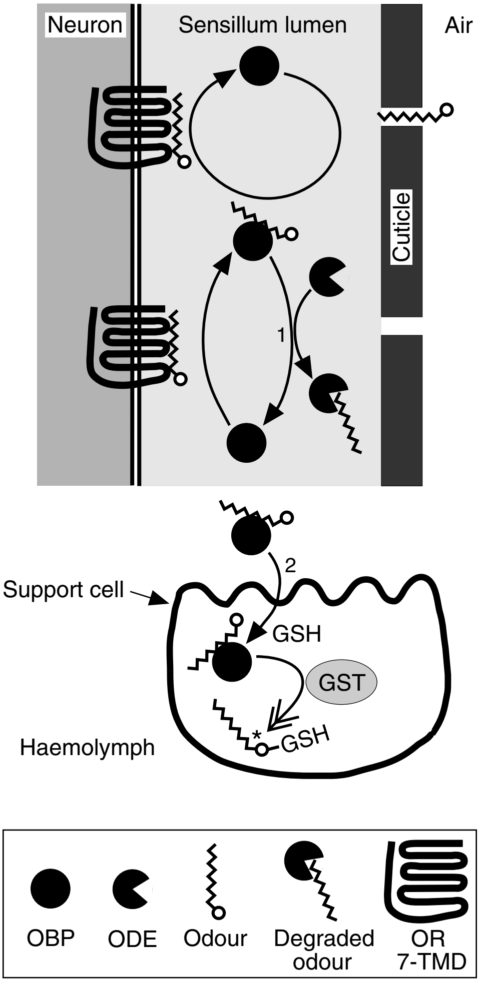


Figure 6.15 Generalized biochemical pathway of odour reception. Hydrophobic odour molecules enter the aqueous sensillum lumen via pores present in the cuticular hair wall. Hydrophilic odour binding proteins (OBPs) supposedly bind and transport odour molecules to receptor proteins (odour receptors (ORs), crossing the receptor neuronal membrane seven times) located in the neuronal membranes. Odour degrading enzymes (ODEs) (pathway 1) in the sensillum lumen supposedly degrade these odour molecules. The cytoplasm of support cells, which surround the nerve cell body, contains xenobiotic inactivating enzymes, such as glutathione-*S*-transferase (GST), which may also serve to inactivate odour molecules (pathway 2). GSH, glutathione; 7-TMD, 7-transmembrane domain. (Modified from Vogt, 2003.)[182]

terminated by odour-degrading enzymes present in the sensillar lymph.[182]

The fruitfly *Drosophila melanogaster* serves as the current model insect species for unravelling the molecular genetic basis of odour detection, as the complete genome sequence has been known since 2000. This insect has 1300 olfactory neurons connected to 43 glomeruli in the antennal lobe. At present, between 25 and 60 candidate OBP genes and 61 candidate seven-transmembrane GPC-OR genes have been reported.[182,183] Although they share particular base sequences, the sequences are diverse, showing only 17–26% sequence conservation and no apparent sequence homology with OR genes in other animal phyla. The numbers of genes implied in *D. melanogaster* are assumed to be similar for other insect species.

Present challenges in insect olfactory transduction are elucidating the functional role of OBPs in olfactory specificity and characterization of the ligand specificity of ORs involved in plant odour recognition.[190] Making use of genomic information on *D. melanogaster* and DNA sequence homology in OR genes of other species, the role of individual OR genes in plant odour recognition can be studied by gene-silencing techniques.[64]

6.5.3 Olfactory electrophysiology and sensitivity

Basically two electrophysiological techniques are employed in studying sensitivity and specificity of the olfactory system in insects. A reflection of simultaneously occurring receptor potentials generated in the entire population of antennal olfactory neurons can be recorded as the so-called electroantennogram (EAG) (Appendix C3). The second method is to record from individual sensilla (so-called single-sensillum or single-cell recording), yielding patterns of action potentials, also called spike activity. This is the actual information-carrying signal that is processed in the CNS. Both methods have their advantages and limitations. The EAG reflects the response of the entire olfactory neuron population but has limited sensitivity. The SCR offers high sensitivity of detection of olfactory activity but in practice allows recording only from a small sample of the entire antennal neuron population.[185]

Like most sensory cells, chemoreceptors are especially responsive to changes in stimulus intensity (i.e. changes in the concentrations of chemicals). Two reaction types occur: excitation, an increase in the rate at which action potentials are produced upon stimulation with an odour; and inhibition, a decrease in firing rate relative to the unstimulated condition, in which spontaneous spiking occurs (Fig. 6.16).

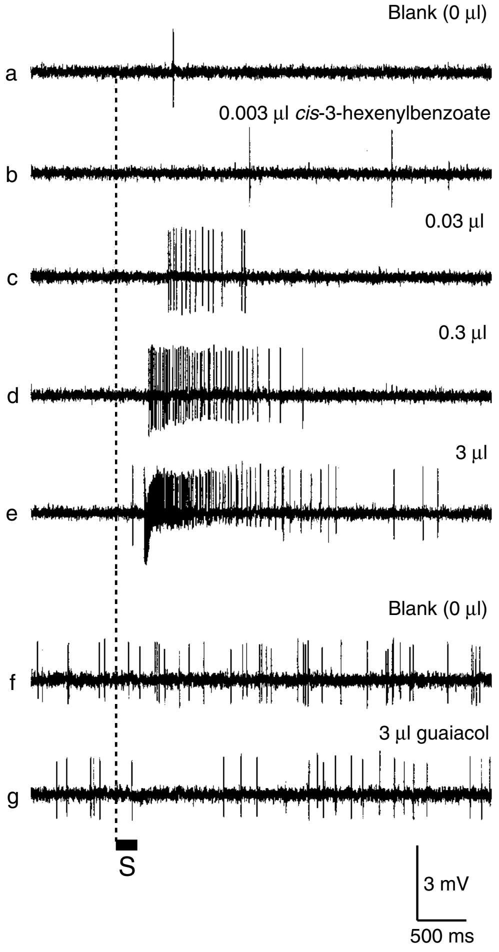


Figure 6.16 Electrophysiological recordings from two different type-A trichoid sensilla on the antenna of a female hawk moth (*Manduca sexta*), showing excitatory (b–e) and inhibitory (g) responses. The hairs were stimulated with the gaseous phase from olfactometer syringes containing filter paper charged with 30 μl of mineral oil solution containing the odorants mentioned. (a–e) Responses of one olfactory receptor neuron (ORN) in a sensillum to: (a) mineral oil alone (blank); (b) 0.003 μl; (c) 0.03 l; (d) 0.3 μl, and (e) 3 μl *cis*-3-hexenylbenzoate, an aromatic ester. (f–g) Responses of one ORN in a different sensillum to: (f) mineral oil alone (blank); (g) 3 μl guaiacol, an aromatic alhohol. Stimulus bar (S) = 200 ms. (From Shields and Hildebrand, 2001.)[148]

Olfactory cells have been shown to handle up to 33 odour pulses per second,[14,110] allowing them to resolve the temporal pattern of odour bursts in a plume (see Fig. 6.5).

Concentration–response relationships generally show a sigmoidal shape at the level of EAGs as well as single-cell recordings (Figs. 6.17 and 6.18). Upon increasing the odour concentration by one order of magnitude, EAG amplitude and frequency of action potentials typically become 1.5–3 times higher until saturating concentrations are reached, above which no further increase occurs. The discrimination of concentration differences is optimal in the range between threshold and saturating concentrations (i.e. the rising phase of the dose–response curves) (Figs. 6.17 and 6.18). This, in principle, enables the insect to sense odour gradients, on the basis of which it may perform tropotactic behaviour (see Section 6.4.3). Different from gustatory receptors, olfactory receptors may function as flux detectors, which track the abundance of molecules over time, rather than concentration detectors.[167]

Sensitivity of detection is enhanced enormously by the neural phenomenon of *convergence*. The

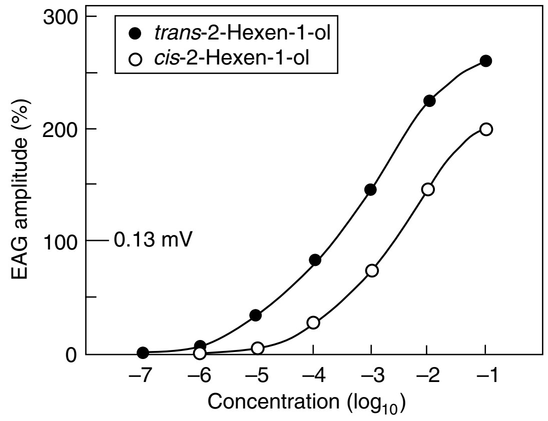


Figure 6.17 Relationship between concentration of two green-leaf volatiles and electroantennogram (EAG) response intensity evoked in the antennae of female Colorado potato beetles. Concentration is expressed as the dilution (v/v) in paraffin oil. EAG response is expressed relative to the response to a standard dose (10^{-3} or 1 μl/ml) of another green-leaf volatile, *cis*-3-hexen-1-ol. The *trans* compound evokes responses at concentrations about 10 times lower than those of the *cis* compound. (From Visser, 1976.)[176]

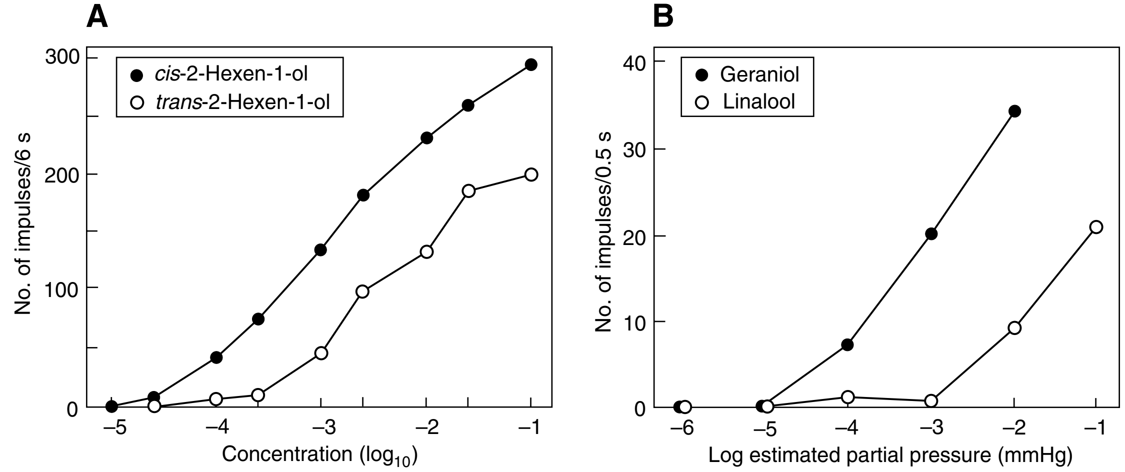


Figure 6.18 Relationship between the concentration of plant volatiles and the responses of single olfactory receptor neurons in two insect species. (**A**) Dose–response relationships for a single olfactory neuron innervating a sensillum basiconicum on the antenna of a female Colorado potato beetle when stimulated with two green-leaf volatiles. Concentration is expressed as the dilution (v/v) in paraffin oil at the source. (From Ma and Visser, 1978.)[98] (**B**) Dose–response relationships for three different olfactory cells narrowly tuned to terpenoid odorants in the antenna of female *Manduca sexta* moths. Vapour pressures of the odorants have been taken into consideration. (From Shields and Hildebrand, 2001.)[148] Note the scale differences in the vertical axes.

axons running from olfactory receptors cells make synaptic contacts with a limited number of first-order interneurons in the antennal lobe of the deuterocerebrum, that is, they converge.[78] A local interneuron receives inputs from many receptor cells simultaneously and its threshold for depolarization may therefore be reached at a lower concentration than that necessary to depolarize a given antennal receptor cell. Convergence improves the signal-to-noise ratio, noise being the spontaneous background activity of the peripheral olfactory system. For example, 100–1000-fold lower concentrations are needed to measure responses in deuterocerebral interneurons to antennal stimulation with green-leaf volatiles in the Colorado potato beetle, compared with thresholds of its antennal receptors.[41] Axons of the olfactory neurons make synaptic contacts in spherical neuropils in the antennal lobe, called glomeruli. A glomerulus is a small convoluted mass of synaptic contacts between olfactory neurons, local interneurons, and projection neurons (Fig. 6.19).[7] In several moth and butterfly species, between 60 and 70 glomeruli are present, in the honeybee 166, and in locusts 1000 glomeruli-like structures have been documented.

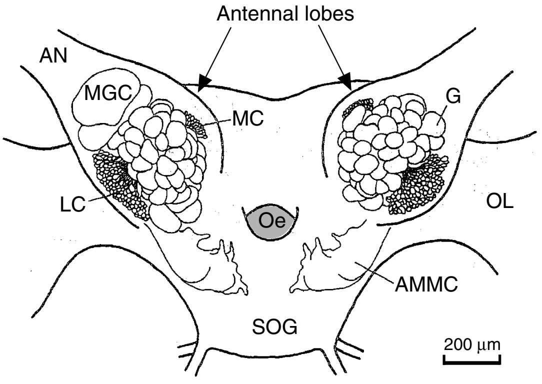


Figure 6.19 Frontal view of the brain of a male sphinx moth (*Manduca sexta*), showing the two neuropils of the deutocerebrum, the antennal lobes, and the antennal mechanosensory and motor centres (AMMC). The macroglomerular complex (MGC) is present only in males. Most cell bodies of antennal lobe interneurons are concentrated in two cell groups, a medial (MC) and a lateral (LC) cluster. AN, antennal nerve; G, glomerulus; SOG, suboesophageal ganglion; Oe, oesophageal canal; OL, optic lobe. (From Anton and Homberg, 1999.)[7]

When the number of projection neurons that arborize in the glomeruli and that send their axons to other brain centres, such as the mushroom bodies, are compared with the number of antennal olfactory cells, a convergence ratio can be calculated, higher values of which are presumably associated with higher sensitivity. For *Locusta migratoria* this ratio is 150, for the honeybee *Apis mellifera* 650, and for the sphinx hawkmoth *Manduca sexta* 330. Antennal lobe output neurons in the brain of *M. sexta* were found to code fine-scale temporal variation in odour intensity in the millisecond domain.[175]

6.5.4 Olfactory specificity and coding

How do olfactory receptors encode the multitude of volatile chemical stimuli present in the outside world into a message that will increase the chance of finding a host plant? Single-cell recording is required to analyse olfactory specificity. Individual plant chemicals and their mixtures can be tested for their effect in evoking changes in chemosensory activity, either excitating or inhibiting olfactory neuron activity. The olfactory system functions as a filter because olfactory receptor neurons are sensitive to only a limited array of volatile chemicals occurring in the environment. For both olfactory and gustatory neurons (see Chapter 7), classically two main categories have been distinguished: 'specialist' and 'generalist' receptor neurons. By definition, a specialist cell responds to only a small number of structurally related compounds, whereas a generalist neuron responds to a wide array of structurally unrelated compounds. Among insect olfactory receptors, sex pheromone receptors are the classical example of specialist receptor.[144]

Over the past decade our view of olfactory neuron specificity in response to plant odours has gradually changed. A growing number of studies on insect herbivores have revealed a hitherto undiscovered degree of specificity of olfactory neurons responding to generally occurring plant volatiles such as green-leaf volatiles and terpenoids in beetles and moths.[5,74,159] On the other hand, olfactory neurons specifically tuned to host-plant specific volatiles have also been found in, for instance, coleopterans,[22] lepidopterous larvae,[178] and aphids.[118] It now seems that generalist receptor neurons are relatively rare. This change of view has been explained by the fact that often, in the past, too-high stimulus concentrations (relative to concentrations prevailing naturally in the environment) were used and, second, by a lack of knowledge of the key stimuli for the specialized olfactory neurons.[74]

Recent findings indicate that oligophagous as well as polyphagous species have olfactory receptor neurons with high sensitivity and selectivity to chemicals that are common in many plant species as well as to chemicals more specific for certain plant groups.[113] Thus, the majority (80%) of olfactory receptor neurons of polyphagous heliothine moths show selective and sensitive responses to the generally occurring sesquiterpenoid (−)-germacrene D, and this compound stimulates oviposition. However, it is unknown what message the airborne concentrations of this particular compound tells about a potential oviposition host plant in the context of all other volatile compounds released simultaneously.

The application of molecular techniques in the analysis of olfactory neuron specificity has led to the current paradigm that one olfactory neuron expresses one receptor protein.[72] An individual receptor protein may interact with structurally diverse volatile ligands. Some ligands activate only few receptors, whereas others activate several receptor types. A single receptor type may produce an excitatory or an inhibitory response, depending on the ligand. It is customary to designate the specificity of a neuron in terms of the molecule to which it shows the lowest threshold[167] and is said to be tuned to this type of (or class of) molecules.

Olfactory receptor neurons can be classified into different response types. Three response types were found in antennal receptors of the sphinx moth *Manduca sexta*[148] and the eucalyptus wood borer *Phoracantha semipunctata*,[12] five were distinguished for antennal receptors of the Colorado potato beetle[98] and the cabbage white butterfly,[43] 12 in the weevil *Pissodes notatus*,[20] and 16 in *Drosophila melanogaster*.[39] The number of response types found will depend on the panel of odorants tested and the size of the antennal neuron population sampled. An organizational feature of the peripheral olfactory system that has received increasing attention is co-compartmentation of olfactory neurons with different specificity in the same sensillum. This has the advantage that the blend ratios between volatile compounds to which the neurons are tuned are perceived and transmitted in an accurate way.[167]

Research into olfactory coding has been extended from classifying response types of antennal olfactory neurons to unravelling olfactory information processing in the antennal lobe and the protocerebrum.[31,78,113] Optical imaging techniques that make use of confocal laser scanning microscopy and calcium-sensitive fluoresecent dyes allow the real-time monitoring of activation patterns of glomeruli when single compounds or mixtures are offered to the antennal receptors.[67,68,151] Based on results obtained with these sophisticated techniques, three-dimensional maps of glomeruli can be constructed[18,95] that allow a spatial representation of odours at the level of identified glomeruli.[18,66,140] It has been found that antennal olfactory neurons of the same functional type project their axons to the same glomerulus, and glomeruli thus function as separate processing units.[31,113] Electrophysiological analyses of projection neurons that transmit olfactory information from a single glomerulus to the mushroom bodies and other protocerebral centres suggest that each glomerulus has a characteristic molecular receptive range[92] and that more than one glomerulus can be involved in processing information on single plant compounds.[31,140]

In the natural environment behaviourally relevant odour signals are always blends. The olfactory system has to encode information on plant odour quality,[31] quantity (concentrations, ratios), and spatial distribution, and to translate it into adequate behavioural decisions.

'Labelled-line' codes have been inferred to operate in oligophagous species, in which the activity of narrowly tuned olfactory neurons may trigger kinetic responses or odour-induced anemotaxis, either positive or negative. 'Across-fibre pattern' codes, supposedly more common in generalist species, are operating through reading out the ratio of the simultaneous activity of a number of olfactory neurons with overlapping but not identical molecular receptive ranges. The cellular elements involved in the analysis of across-fibre patterning are local interneurons and projection neurons of the antennal lobe. Although both coding modes are sometimes described as mutually exclusive, they more likely represent extremes of a continuum.[152] As many plant species release a complex blend of generally occurring green-leaf volatiles and terpenoids into the atmosphere (see Chapter 4) that lack qualitative taxonomic specificity, neural coding of ratios of the quantities released becomes critical, as these ratios may contain information on the plant taxon.[177] Across-fibre or combinatorial codes are better suited for this purpose and require fewer receptors to accomplish this task.[23]

At the behavioural level, generally occurring green-leaf volatiles may synergize with one another, and also with taxonomically specific volatiles or with pheromones.[27,38,60] Likewise, at the olfactory receptor level, interactions have been shown to occur between host-plant odour components as well as between host-plant odours and pheromones.[73,119,173,180]

Aspects of olfactory specificity, coding principles, and CNS processing of plant odour information are rapidly evolving areas of study. These studies focus on a number of model species: the honeybee *Apis mellifera*,[67] *Spodoptera* spp.,[140] *Helicoverpa* spp.,[151] and *Manduca sexta*.[148]

6.6 Host-plant searching in nature

When an herbivorous insect is searching for a host plant in the field, it meets a multitude of stimuli, which are distributed heterogeneously. Inherent to the field situation is a lack of control over both the stimulus situation and abiotic parameters that possibly influence behavioural responses. It is therefore difficult to assess the relative importance of the two main stimulus modalities, optical and odorous plant cues, under field conditions. For several insect species it has been shown that significant stimulus interactions occur. During searching for food or oviposition sites, the importance of different types of stimulus may change with distance to the plant. Stimulus interactions may be one of the causes of the discrepancies indicated in Table 6.3, for which behavioural responses to odours observed in the laboratory could not be confirmed in the field.

The Colorado potato beetle, for instance, is well able to perform directed orientation in response to odours alone and uses odour-conditioned positive anemotaxis, as has been convincingly demonstrated in laboratory studies.[162,176] Behavioural observations on host-plant searching in the field, however, have given variable results with respect to the role of odours in host-plant location. De Wilde found upwind menotactic responses in the field at distances less than 6 m from a plot of potato plants (of unstated size).[49] Jermy and co-workers, however, found only a low proportion of beetles moving upwind in the field, and even in these cases their walking tracks did not reveal directed movement towards potato plants.[83] The beetles showed photomenotaxis and a high directionality of movement based on light-compass orientation rather than on odour-induced anemotaxis. In the vicinity of potato plants, interruptions of straight paths occurred, accompanied by an increased rate of turning. Jermy and co-workers estimated that the maximum distance at which a walking beetle could detect a single potato plant was about 60 cm, based on either olfactory cues or visual cues, or a combination. However, only one of every two beetles that came within this radius of detection was attracted to the plant. Odour masking is likely to be one of the causes of the small radius of detection in a complex natural vegetation. It was concluded that, under natural conditions, where individual potato plants may be scattered between non-hosts, host-plant finding is a chance event when the beetle starts at a distance of more than 60 cm from a potato plant.[83] These findings fit well into the model of 'alternating random and non-random (kinetic arrestment-type) search strategies' formulated by Morris and Kareiva.[107]

Of all herbivorous insects in which host-searching behaviour has been studied, the apple maggot fly *Rhagoletis pomonella* and the cabbage root fly *Delia radicum* are probably those analysed in most detail.[3,4,62,139] The visually guided host-searching behaviour of the apple maggot fly was described above (see Section 6.4.2). These flies are highly responsive to particular visual stimuli, but only after they have been 'activated' by apple odour. They show preferences for either yellow or red, depending on the size of the object and their motivational state (see Section 6.2). Spherical red objects of a limited diameter are preferred when the fly is searching for ovipostion sites. In order to acquire carbohydrates, the flies feed on aphid honeydew, which is present on apple leaves. Larger yellow spheres are preferred over red ones when the motivation for carbohydrate ingestion is high. Yellow serves as a supernormal substitute stimulus for the green hue of apple leaves. Apple odour elicits upwind flight, and odour-induced anemotaxis allows the flies to locate an apple-bearing tree within a patch of trees devoid of apples by a series of tree-to-tree displacements. In the same way they can find a synthetic odour source outside an odourless patch. Once at a tree bearing apples, selection of individual fruits by size or colour is done mainly visually. However, when there are few fruits or when they are green instead of red and therefore lack contrast with the leaves, odorous cues are used to aid the selection process (Fig. 6.20).

As an alternative to seven existing hypotheses, a new hypothesis to explain how plant diversity affects host-plant selection behaviour was launched by Finch and Collier.[62] It is based on a substantial amount of detailed behavioural observations on *Delia* flies and other insects associated with cruciferous plants. Finch and Collier propose that selection occurs in three phases, the first governed by

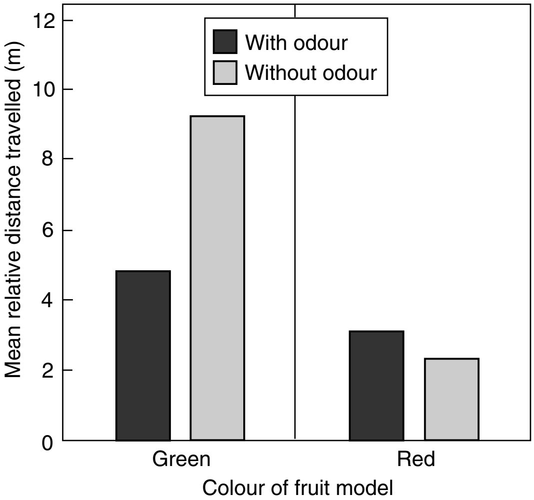


Figure 6.20 Demonstration of the interaction between olfactory and visual information in host selection behaviour of the apple maggot fly *Rhagoletis pomonella*; the effect of host fruit odour (a synthetic blend of six esters), released at 500 mg/h, on finding green or red fruit models in a tree carrying 16 models. (From Aluja and Prokopy, 1993.)[3]

volatile plant cues, the next by visual cues, and the final phase largely affected by non-volatile plant chemicals (see Chapter 7). The second phase has been largely overlooked and can be described in terms of indiscriminate landings on appropriate (host) plants and inappropriate (non-host) plants. In the first phase, olfaction of plant volatiles leads to arrestment and landing but provides no directional information (see Section 6.3). In the second phase, repeated contacting of the host plant is crucial to reinforce the stimulatory information that leads to entering the third phase, in which oviposition or feeding decisions are based largely, although not exclusively in the case of *Delia*,[40] on contact cues.

Field studies to date on host plant-searching behaviour have logically been focused on larger species, which, by virtue of their visual conspicuousness, can be directly observed and followed for some time while moving from plant to plant. As a result oviposition behaviour of butterflies has been studied in some detail.[9,150] The picture that emerges from these studies is a predominant role of vision and associative learning involving optical and contact-chemosensory cues, promoting time and energy optimization of host selection behaviour.

In contrast to the situation mentioned above for fruit flies, host selection behaviour of bark beetles in forest ecosystems is governed largely by chemical cues. Highly intricate chemical communication systems are operating based on complicated interactions between host-tree odours, aggregation pheromones produced by the beetles or associated microorganisms, and interspecific inhibitory infochemicals.[25,103,131]

6.7 Conclusions

Although our knowledge of the plant characteristics influencing host-plant searching and the ways in which insect herbivores detect and use them to their own advantage is increasing, the general picture is built upon information from a small number of relatively well studied species. Clearly, plant factors that affect insects over some distance are difficult to manipulate experimentally in the field. In many specialized herbivores no evident orientation can be demonstrated when they are at some distance from their host plant, and it appears that in order to find a suitable plant they must literally bump into it. Searching then is essentially a random process, in which the chance of an encounter is determined largely by spatial factors.[30,44,83] However, several specialized insect species have been observed under natural conditions to be perfectly able to integrate information from different cues, and studies have shown that the outcome of this integration, manifested as searching behaviour, is more complex than expected from a mere summation of responses across sensory modalities.[17,33,75]

New insights in the molecular biology and neurophysiology of odour detection have evolved rapidly over the past decade and have significantly increased our understanding of the mechanisms involved. The challenge is to relate knowledge at the molecular and physiological levels to the behavioural and evolutionary significance of using odour information. Plant headspace volatile composition is complex in qualitative and quantitative respects, yet it is essential, when investigating which compounds convey relevant information about the suitability of a host plant, to know their naturally occurring concentrations. In fact, the minimal blend of identified volatiles causing attraction has been successfully formulated for relatively few species.[102,155]

It can be expected that in the near future an integration of molecular biology, neurobiology, behaviour, and phytochemistry will considerably advance our understanding of host plant-finding mechanisms and the evolutionary selection pressures that mould them.[113] In addition, it appears fruitful to pay explicit attention to the integration of optical and odorous plant cues.[62]

6.8 References

1. Ahmad, S. (1982). Host location by the Japanese beetle: evidence for a key role for olfaction in a highly polyphagous insect. *Journal of Experimental Zoology*, **220**, 117–20.
2. Aikman, D. and Hewitt, G. (1972). An experimental investigation of the rate and form of dispersal in grasshoppers. *Journal of Applied Ecology*, **9**, 807–17.

3. Aluja, M. and Prokopy, R. (1993). Host odor and visual stimulus interactions during intratree host finding behavior of *Rhagoletis pomonella* flies. *Journal of Chemical Ecology*, **19**, 2671–96.
4. Aluja, M., Prokopy, R.J., Buonaccorsi, J.P., and Cardé, R.T. (1993). Wind tunnel assays of olfactory responses of female *Rhagoletis pomonella* flies to apple volatiles: effect of wind speed and odour release rate. *Entomologia Experimentalis et Applicata*, **68**, 99–108.
5. Anderson, P., Hansson, B.S., and Löfquist, J. (1995). Plant-volatile receptor neurons on the antennae of female and male *Spodoptera littoralis. Physiological Entomology*, **20**, 189–98.
6. Ansebo, L., Coracini, M.D.A., Bengtsson, M., Liblikas, I., Ramírez, M., Borg-Karlson, A.-K., *et al.* (2004). Antennal and behavioural response of codling moth *Cydia pomonella* to plant volatiles. *Journal of Applied Entomology*, **128**, 488–93.
7. Anton, S. and Homberg, U. (1999). Antennal lobe structure. In *Insect olfaction* (ed. B.S. Hansson), pp. 97–124. Springer, Berlin.
8. Atkins, M.D. (1980). *Introduction to insect behavior*. Macmillan, New York.
9. Auckland, J.N., Debinski, D.M., and Clark, W.R. (2004). Survival, movement, and resource use of the butterfly *Parnassius clodius. Ecological Entomology*, **29**, 139–49.
10. Baker, T.C. (1988). Pheromones and flight behavior. In *Insect flight* (ed. G. Goldsworthy and C. Wheeler), pp. 231–55. CRC Press, Boca Raton, FL.
11. Balkovsky, E. and Shraiman, B.I. (2002). Olfactory search at high Reynolds numbers. *Proceedings of the National Academy of Sciences of the USA*, **99**, 12589–93.
12. Barata, E.N., Mustaparta, H., Pickett, J.A., Wadhams, L.J., and Araujo, J. (2002). Encoding of host and non-host plant odours by receptor neurones in the eucalyptus woodborer, *Phoracantha semipunctata* (Coleoptera: Cerambycidae). *Journal of Comparative Physiology A*, **188**, 121–33.
13. Barton Browne, L. (1993). Physiologically induced changes in resource oriented behavior. *Annual Review of Entomology*, **38**, 1–25.
14. Bau, J., Justus, K.A., and Cardé, R.T. (2002). Antennal resolution of pulsed pheromone plumes in three moth species. *Journal of Insect Physiology*, **48**, 433–42.
15. Baur, R., Feeny, P., and Städler, E. (1993). Oviposition stimulants for the black swallowtail butterfly: identification of electrophysiologically active compounds in carrot volatiles. *Journal of Chemical Ecology*, **19**, 919–37.
16. Bell, W.J. (1984). Chemo-orientation in walking insects. In *Chemical ecology of insects* (ed. W.J. Bell and R.T. Cardé), pp. 93–109. Chapman & Hall, London.
17. Bell, W.J. (1991). *Searching behaviour. The behavioural ecology of finding resources*. Chapman & Hall, London.
18. Berg , B.G., Galizia, C.G., Brandt, R., and Mustaparta, H. (2002). Digital atlases of the antennal lobe in two species of tobacco budworm moths, the oriental *Helicoverpa assulta* (male) and the American *Heliothis virescens* (male and female). *Journal of Comparative Neurology*, **446**, 123–34.
19. Bernays, E.A. and Chapman, R.F. (1994). *Host-plant selection by phytophagous insects*. Chapman & Hall, New York.
20. Bichao, H., Borg-Karlson, A.K., Araujo, J., and Mustaparta, H. (2003). Identification of plant odours activating receptor neurones in the weevil *Pissodes notatus* F. (Coleoptera, Curculionidae). *Journal of Comparative Physiology A*, **189**, 203–12.
21. Blaakmeer, A., Geervliet, J.B.F., Van Loon, J.J.A., Posthumus, M.A., Van Beek, T.A., and De Groot, A.E. (1994). Comparative headspace analysis of cabbage plants damaged by two species of *Pieris* caterpillars: consequences for inflight host location by *Cotesia* parasitoids. *Entomologia Experimentalis et Applicata*, **73**, 175–82.
22. Blight, M.M., Pickett, J.A., Wadhams, L.J., and Woodcock, C.M. (1989). Antennal responses of *Ceutorhynchus assimilis* and *Psylliodes chrysocephala* to volatiles from oilseed rape. *Aspects of Applied Biology*, **23**, 329–34.
23. Boeckh, J. and Ernst, K.D. (1983). Olfactory food and mate recognition. In *Neuroethology and behavioral physiology* (ed. F. Huber and H. Markl), pp. 78–94. Springer, Berlin.
24. Briscoe, A.D. and Chittka, L. (2001). The evolution of color vision in insects. *Annual Review of Entomology*, **46**, 471–510.
25. Byers, J.A. (1995). Host-tree chemistry affecting colonization of bark beetles. In *Chemical ecology of insects*, Vol. 2 (ed. R.T. Cardé and W.J. Bell), pp. 154–213. Chapman & Hall, New York.
26. Byers, J.A., Anderbrant, O., and Löfqvist, J. (1989). Effective attraction radius: a method for comparing species attractants and determining densities of flying insects. *Journal of Chemical Ecology*, **15**, 749–65.
27. Byers, J.A., Birgersson, G., Löfqvist, J., Appelgren, M., and Bergström, G. (1990). Isolation of pheromone synergists of bark beetle, *Pityogenes chalcographus*, from complex insect-plant odors by fractionation and subtractive-combination bioassay. *Journal of Chemical Ecology*, **16**, 861–76.
28. Campbell, C.A.M., Pettersson, J., Pickett, J.A., Wadhams, L.J., and Woodcock, C.M. (1993). Spring

migration of damsonhop aphid *Phorodon humuli* (Homoptera, Aphididae), and summer host plant-derived semiochemicals released on feeding. *Journal of Chemical Ecology*, **19**, 1569–76.

29. Cantelo, W.W. and Jacobson, M. (1979). Corn silk volatiles attract many pest species of moths. *Journal of Environmental Science and Health A*, **14**, 695–707.
30. Cappuccino, N. and Kareiva, P.M. (1985). Coping with capricious environment: a population study of a rare pierid butterfly. *Ecology*, **66**, 152–61.
31. Carlsson, M.A. and Hansson, B.S. (2003). Plasticity and coding mechanisms in the insect antennal lobe. In *Insect pheromone biochemistry and molecular biology* (ed. G.J. Blomquist and R.G. Vogt), pp. 699–728. Elsevier, Amsterdam.
32. Chapman, R.F. (1982). Chemoreception: the significance of sensillum numbers. *Advances in Insect Physiology*, **16**, 247–356.
33. Chapman, R.F. (1988). Odors and the feeding behavior of insects. *ISI Atlas of Science: Animal and Plant Sciences*, pp. 208–12.
34. Chapman, R.F., Bernays, E.A., and Simpson, S.J. (1981). Attraction and repulsion of the aphid, *Cavariella aegopodii*, by plant odors. *Journal of Chemical Ecology*, **7**, 881–8.
35. Chittka, L. and Waser, N.M. (1997). Why red flowers are not invisible to bees. *Israel Journal of Plant Sciences*, **45**, 169–83.
36. Clyne, P.J., Warr, C.G., and Carlson, J.R. (2000). Candidate taste receptors in *Drosophila*. *Science*, **287**, 1830–4.
37. Coombe, P.E. (1982). Visual behaviour of the greenhouse whitefly *Trialeurodes vaporariorum*. *Physiological Entomology*, **7**, 243–51.
38. Coracini, M., Bengtsson, M., Liblikas, I., and Witzgall, P. (2004). Attraction of codling moth males to apple volatiles. *Entomologia Experimentalis et Applicata*, **110**, 1–10.
39. De Bruyne, M., Foster, K., and Carlson, J.R. (2001). Odor coding in the *Drosophila* antenna. *Neuron*, **30**, 537–52.
40. De Jong, R. and Städler, E. (1999). The influence of odour on the oviposition behaviour of the cabbage root fly. *Chemoecology*, **9**, 151–4.
41. De Jong, R. and Visser, J.H. (1988). Integration of olfactory information in the Colorado potato beetle brain. *Brain Research*, **447**, 10–17.
42. De Moraes, C.M., Mescher, M.C., and Tumlinson, J.H. (2001). Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature*, **410**, 577–80.
43. Den Otter, C.J., Behan, M., and Maes, F.W. (1980). Single cell responses in female *Pieris brassicae* (Lep.: Pieridae) to plant volatiles and conspecific egg odours. *Journal of Insect Physiology*, **26**, 465–72.
44. Dethier, V.G. (1959). Foodplant distribution and density and larval dispersal as factors affecting insect populations. *Canadian Entomologist*, **91**, 581–96.
45. Dethier, V.G. (1982). Mechanisms of host plant recognition. *Entomologia Experimentalis et Applicata*, **31**, 49–56.
46. Dethier, V.G. (1989). Patterns of locomotion of polyphagous arctiid caterpillars in relation to foraging. *Ecological Entomology*, **14**, 375–86.
47. Dethier, V.G. and Schoonhoven, L.M. (1968). Evaluation of evaporation by cold and humidity receptors in caterpillars. *Journal of Insect Physiology*, **14**, 1049–54.
48. Dethier, V.G., Barton Browne, L., and Smith, C.N. (1960). The designation of chemicals in terms of the responses they elicit from insects. *Journal of Economic Entomology*, **53**, 134–6.
49. De Wilde, J. (1976). The olfactory component in host-plant selection in the adult Colorado beetle (*Leptinotarsa decemlineata* Say). *Symposia Biologica Hungarica*, **16**, 291–300.
50. Dicke, M. and Sabelis, M.W. (1988). Infochemical terminolgy: based on cost–benefit analysis rather than origin of compounds? *Functional Ecology*, **2**, 131–9.
51. Dicke, M. and Van Loon, J.J.A. (2000). Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata*, **97**, 237–49.
52. Dickens, J.C. (1984). Olfaction in the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae): electroantennogram studies. *Journal of Chemical Ecology*, **10**, 1759–85.
53. Dickens, J.C. (1986). Orientation of boll weevil *Anthonomus grandis* Boh. (Coleoptera: Curculionidae), to pheromone and volatile host compound in the laboratory. *Journal of Chemical Ecology*, **12**, 91–8.
54. Dickens, J.C. (1990). Specialized receptor neurons for pheromones and host plant odors in the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae). *Chemical Senses*, **15**, 311–31.
55. Douwes, P. (1968). Host selection and host finding in the egg-laying female *Cidaria albulata* L. (Lep. Geometridae). *Opuscula Entomologica*, **33**, 233–79.
56. Drew, R.A.I., Prokopy, R.J., and Romig, M.C. (2003). Attraction of fruit flies of the genus *Bactrocera* to colored mimics of host fruit. *Entomologia Experimentalis et Applicata*, **107**, 39–45.
57. Eigenbrode, S.D., Ding, H., Shiel, P., and Berger, P.H. (2002). Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus

vector, *Myzus persicae* (Homoptera: Aphididae). *Proceedings of the Royal Society of London, Series B*, **269**, 455–60.

58. Evans, K.A. and Allen-Williams, L.J. (1993). Distant olfactory response of the cabbage seed weevil, *Ceutorhynchus assimilis*, to oilseed rape odour in the field. *Physiological Entomology*, **18**, 251–6.
59. Fadamiro, H.Y., Wyatt, T.D., and Birch, M.C. (1998). Flying beetles respond as moths predict: optomotor anemotaxis to pheromone plumes at different heights. *Journal of Insect Behavior*, **11**, 549–57.
60. Fein, B.L., Reissig, W.H., and Roelofs, W.L. (1982). Identification of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella*. *Journal of Chemical Ecology*, **8**, 1473–87.
61. Finch, S. (1986). Assessing hostplant finding by insects. In *Insect–plant interactions* (ed. J.R. Miller and T.A. Miller), pp. 23–63. Springer, New York.
62. Finch, S. and Collier, R.H. (2001). Host-plant selection by insects—a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. *Entomologia Experimentalis et Applicata*, **96**, 91–102.
63. Finch, S. and Skinner, G. (1982). Upwind flight by the cabbage root fly, *Delia radicum*. *Physiological Entomology*, **7**, 387–99.
64. Fire, A. (1999). RNA-triggered gene silencing. *Trends in Genetics*, **15**, 358–63.
65. Frey, J.E. and Bush, G.L. (1990). Rhagoletis sibling species and host races differ in host odor recognition. *Entomologia Experimentalis et Applicata*, **57**, 123–31.
66. Galizia, C.G. and Menzel, R. (2000). Odour perception in honeybees: coding information in glomerular patterns. *Current Opinion in Neurobiology*, **10**, 504–10.
67. Galizia, C.G. and Menzel, R. (2001). The role of glomeruli in the neural representation of odours: results from optical recording studies. *Journal of Insect Physiology*, **47**, 115–30.
68. Galizia, C.G., Sachse, S., and Mustaparta, H. (2000). Calcium responses to pheromones and plant odours in the antennal lobe of the male and female moth *Heliothis virescens*. *Journal of Comparative Physiology A*, **186**, 1049–63.
69. Getz, W.M. and Akers, R.P. (1994). Honeybee olfactory sensilla behave as integrated processing units. *Behavioral and Neural Biology*, **61**, 191–5.
70. Guerin, P.M. and Städler, E. (1982). Host odour perception in three phytophagous Diptera—a comparative study. In *Proceedings of the 5th international symposium on insect–plant relationships, Wageningen, 1982* (ed. J.H. Visser and A.K. Minks), pp. 95–105. Pudoc, Wageningen.
71. Guerin, P.M. and Städler, E. (1984). Carrot fly cultivar preferences: some influencing factors. *Ecological Entomology*, **9**, 413–20.
72. Hallem, E.A., Ho, M.G., and Carlson, J.R. (2004). The molecular basis of odor coding in the *Drosophila* antenna. *Cell*, **117**, 965–79.
73. Hansson, B.S., Van der Pers, J.N.C., and Löfqvist, J. (1989). Comparison of male and female olfactory cell response to pheromone compounds and plant volatiles in the turnip moth, *Agrotis segetum*. *Physiological Entomology*, **14**, 147–55.
74. Hansson, B.S., Larsson, M.C., and Leal, W.S. (1999). Green leaf volatile-detecting olfactory receptor neurones display very high sensitivity and specificity in a scarab beetle. *Physiological Entomology*, **24**, 121–6.
75. Harris, M.O. and Foster, S.P. (1995). Behavior and Integration. In *Chemical ecology of insects*, Vol. 2 (ed. R.T. Cardé and W.J. Bell), pp. 3–46. Chapman & Hall, New York.
76. Hern, A. and Dorn, S. (2004). A female-specific attractant for the codling moth, *Cydia pomonella*, from apple fruit volatiles. *Naturwissenschaften*, **91**, 77–80.
77. Hesler, L.S., Lance, D.R., and Sutter, G.R. (1994). Attractancy of volatile non-pheromonal semiochemicals to northern corn rootworm beetles (Coleoptera: Chrysomelidae) in eastern South Dakota. *Journal of the Kansas Entomological Society*, **67**, 186–92.
78. Hildebrand, J.G. (1996). Olfactory control of behavior in moths: central processing of odor information and the functional significance of olfactory glomeruli. *Journal of Comparative Physiology A*, **178**, 5–19.
79. Hirota, T. and Kato, Y. (1999). Influence of visual stimuli on host location in the butterfly, *Eurema hecabe*. *Entomologia Experimentalis et Applicata*, **101**, 199–206.
80. Honda, I., Ishikawa, Y. and Matsumoto, Y. (1987). Electrophysiological studies on the antennal olfactory cells of the onion fly, *Hylemya antiqua* Meigen (Diptera: Anthomyiidae). *Applied Entomology and Zoology*, **22**, 417–23.
81. Isaacs, R., Willis, M.A., and Byrne, D.N. (1999). Modulation of whitefly take-off and flight orientation by wind speed and visual cues. *Physiological Entomology*, **24**, 311–18.
82. Jackson, D.M., Severson, R.F., Johnson, A.W., and Herzog, G.A. (1986). Effects of cuticular duvane diterpenes from green tobacco leaves on tobacco budworm (Lepidoptera: Noctuidae). *Journal of Chemical Ecology*, **12**, 1349–59.
83. Jermy, T., Szentesi, Á., and Horváth, J. (1988). Host plant finding in phytophagous insects: the case of the

Colorado potato beetle. *Entomologia Experimentalis et Applicata*, **49**, 83–98.

84. Jönsson, M. and Anderson, P. (1999). Electrophysiological response to herbivore-induced host-plant volatiles in the moth *Spodoptera littoralis*. *Physiological Entomology*, **24**, 377–85.
85. Judd, J.G.R. and Borden, J. (1989). Distant olfactory response of the onion fly, *Delia antiqua*, to host-plant odour in the field. *Physiological Entomology*, **14**, 429–41.
86. Kalberer, N.M., Turlings, T.C.J., and Rahier, M. (2001). Attraction of a leaf beetle (*Oreina cacaliae*) to damaged host plants. *Journal of Chemical Ecology*, **27**, 647–61.
87. Keil, T.A. (1999). Morphology and development of the peripheral olfactory organs. In *Insect olfaction* (ed. B.S. Hansson), pp. 5–47. Springer, Berlin.
88. Kelber, A. (1999). Why 'false' colours are seen by butterflies. *Nature*, **402**, 251.
89. Kennedy, J.S. (1986). Some current issues in orientation to odour sources. In *Mechanisms in insect olfaction* (ed. T.L. Payne, M.C. Birch, and C.E.J. Kennedy), pp. 11–25. Oxford University Press, Oxford.
90. Kennedy, J.S., Booth, C.O., and Kershaw, W.J.S. (1959). Host finding by aphids in the field. II. *Aphis fabae* Scop. (gynoparae) and *Brevicoryne brassicae* L., with a reappraisal of the role of host finding behaviour in virus spread. *Annals of Applied Biology*, **47**, 424–44.
91. Kennedy, J.S., Booth, C.O., and Kershaw, W.J.S. (1961). Host finding by aphids in the field. III. Visual attraction. *Annals of Applied Biology*, **49**, 1–21.
92. King, J.R., Christensen, T., and Hildebrand, J.G. (2000). Response characteristics of an identified, sexually dimorphic olfactory glomerulus. *Journal of Neuroscience*, **20**, 2391–9.
93. Klingler, J. (1958). Die Bedeutung der Kohlendioxyd Ausscheidung der Wurzeln für die Orientierung der Larven von *Otiorhynchus sulcatus* F. und anderer bodenbewohnender phytophager Insektenarten. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft*, **31**, 205–69.
94. Kuenen, L.P.S. and Cardé, R.T. (1994). Strategies for recontacting a lost pheromone plume: casting and upwind flight in the male gypsy moth. *Physiological Entomology*, **19**, 15–29.
95. Laissue, P.P., Reiter, C., Hiesinger, P.R., Halter, S., Fischbach, K.F., and Stocker, R.F. (1999). Three-dimensional reconstruction of the antennal lobe in *Drosophila melanogaster*. *Journal of Comparative Neurology*, **405**, 543–52.
96. Landolt, P.J. (1989). Attraction of the cabbage looper to host plants and host plant odor in the laboratory. *Entomologia Experimentalis et Applicata*, **53**, 117–24.
97. Langer, H., Hamann, B., and Meinecke, C.C. (1979). Tetrachromatic visual system in the moth *Spodoptera exempta* (Insecta: Noctuidae). *Journal of Comparative Physiology*, **129**, 235–9.
98. Ma, W.C. and Visser, J.H. (1978). Single unit analysis of odour quality coding by the olfactory antennal receptor system of the Colorado beetle. *Entomologia Experimentalis et Applicata*, **24**, 520–33.
99. Matsumoto, Y. (1970). Volatile organic sulfur compounds as insect attractants with special reference to host selection. In *Control of insect behavior by natural products* (ed. D.L. Wood, R.M. Silverstein, and M. Nakajima), pp. 133–160. Academic Press, New York.
100. Mechaber, W.L., Capaldo, C.T., and Hildebrand, J.G. (2002). Behavioral responses of adult female tobacco hornworms, *Manduca sexta*, to hostplant volatiles change with age and mating status. *Journal of Insect Science*, **2**(5), 1–8.
101. Meisner, J. and Ascher, K.R.S. (1973). Attraction of *Spodoptera littoralis* larvae to colours. *Nature*, **242**, 332–4.
102. Metcalf, R.L. and Metcalf, E.R. (1992). *Plant kairomones in insect ecology and control*. Chapman & Hall, New York.
103. Miller, D.R. and Borden, J.H. (2000). Dose-dependent and species-specific responses of pine bark beetles (Coleoptera: Scolytidae) to monoterpenes in association with pheromones. *Canadian Entomologist*, **132**, 183–95.
104. Miller, J.R. and Strickler, K.L. (1984). Finding and accepting host plants. In *Chemical ecology of insects* (ed. W.J. Bell and R.T. Cardé), pp. 127–57. Chapman & Hall, London.
105. Moeckh, H.A. (1981). Host selection behavior of bark beetles (Col: Scolytidae) attacking *Pinus ponderosa*, with special emphasis on the western pine beetle, *Dendroctonus brevicomis*. *Journal of Chemical Ecology*, **7**, 49–83.
106. Moericke, V. (1969). Hostplant specific colour behaviour by *Hyalopterus pruni* (Aphididae). *Entomologia Experimentalis et Applicata*, **12**, 524–34.
107. Morris, W.F. and Kareiva, P.M. (1991). How insect herbivores find suitable host plants: the interplay between random and nonrandom movement. In *Insect–plant interactions*, Vol. 3 (ed. E.A. Bernays), pp. 175–208. CRC Press, Boca Raton.

108. Mulkern, G.B. (1969). Behavioral influences on food selection in grasshoppers (Orthoptera: Acrididae). *Entomologia Experimentalis et Applicata*, **12**, 509–23.
109. Murlis, J. (1986). The structure of odour plumes. In *Mechanisms in insect olfaction* (ed. T.L. Payne, M.C. Birch, and C.E.J. Kennedy), pp. 27–38. Oxford University Press, Oxford.
110. Murlis, J., Elkinton, J.S., and Cardé, R.T. (1992). Odor plumes and how insects use them. *Annual Review of Entomology*, **37**, 505–32.
111. Murlis, J., Willis, M.A., and Cardé, R.T. (2000). Spatial and temporal structures of pheromone plumes in fields and forests. *Physiological Entomology*, **25**, 211–22.
112. Mustaparta, H. (1984). Olfaction. In *Chemical ecology of insects* (ed. W.J. Bell and R.T. Cardé), pp. 37–70. Chapman & Hall, London.
113. Mustaparta, H. (2002). Encoding of plant odour information in insects: peripheral and central mechanisms. *Entomologia Experimentalis et Applicata*, **104**, 1–13.
114. Nojima, S., Linn, C., and Roelofs, W. (2003). Identification of host fruit volatiles from flowering dogwood (*Cornus florida*) attractive to dogwood-origin *Rhagoletis pomonella* flies. *Journal of Chemical Ecology*, **29**, 2347–57.
115. Nordenhem, H. and Nordlander, G. (1994). Olfactory oriented migration through soil by root-living *Hylobius abietis* (L.) larvae (Col., Curculionidae). *Journal of Applied Entomology*, **117**, 457–62.
116. Nordlund, D.A. and Lewis, W.J. (1976). Terminology of specific releasing stimuli in intraspecific and interspecific interactions. *Journal of Chemical Ecology*, **2**, 211–20.
117. Nottingham, S.F. (1987). Effects of nonhost-plant odors on anemotactic response to host-plant odor in female cabbage root fly, *Delia radicum*, and carrot rust fly, *Psila rosae. Journal of Chemical Ecology*, **13**, 1313–18.
118. Nottingham, S.F., Hardie, J., Dawson, G.W., Hick, A.J., Pickett, J.A., Wadhams, L.J., *et al.* (1991). Behavioral and electrophysiological responses of aphids to host and nonhost plant volatiles. *Journal of Chemical Ecology*, **17**, 1231–42.
119. Ochieng, S.A., Park, K.C., and Baker, T.C. (2002). Host plant volatiles synergize responses of sex pheromone-specific olfactory receptor neurons in male *Helicoverpa zea. Journal of Comparative Physiology A*, **188**, 325–33.
120. Paliniswamy, P. and Gillot, C. (1986). Attraction of diamondback moths, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), by volatile compounds of canola, white mustard, and faba bean. *Canadian Entomologist*, **118**, 1279–85.
121. Papaj, D.R. and Prokopy, R.J. (1989). Ecological and evolutionary aspects of learning in phytophagous insects. *Annual Review of Entomology*, **34**, 315–50.
122. Pettersson, J. (1970). Studies on *Rhopalosiphum padi* (L.). I. Laboratory studies on olfactometric responses to the winter host *Prunus padus* L. *Lantbrukshoegskolan Annaler*, **36**, 381–99.
123. Pettersson, J. (1973). Olfactory reactions of *Brevicoryne brassicae* (L.) (Hom.: Aph.). *Swedish Journal of Agricultural Research*, **3**, 95–103.
124. Pivnick, K.A., Lamb, R.J., and Reed, D. (1992). Responses of flea beetles, *Phyllotreta* spp., to mustard oils and nitriles in field trapping experiments. *Journal of Chemical Ecology*, **18**, 863–73.
125. Pospisil, J. (1972). Olfactory orientation of certain phytophagous insects in Cuba. *Acta Entomologica Bohemoslovaca*, **69**, 7–17.
126. Prokopy, R.J. (1968). Visual responses of apple maggot flies, *Rhagoletis pomonella* (Diptera: Tephritidae): orchard studies. *Entomologia Experimentalis et Applicata*, **11**, 403–22.
127. Prokopy, R.J. (1986). Visual and olfactory stimulus interaction in resource finding by insects. In *Mechanisms in insect olfaction* (ed. T.L. Payne, M.C. Birch, and C.E.J. Kennedy), pp. 81–9. Oxford University Press, Oxford.
128. Prokopy, R.J. and Owens, E.D. (1983). Visual detection of plants by herbivorous insects. *Annual Review of Entomology*, **28**, 337–64.
129. Prokopy, R.J., Collier, R., and Finch, S. (1983a). Leaf color used by cabbage root flies to distinguish among host plants. *Science*, **221**, 190–2.
130. Prokopy, R.J., Collier, R., and Finch, S. (1983b). Visual detection of host plants by cabbage root flies. *Entomologia Experimentalis et Applicata*, **34**, 85–9.
131. Reddy, G.V.P. and Guerrero, A. (2004). Interactions of insect pheromones and plant semiochemicals. *Trends in Plant Science*, **9**, 253–61.
132. Roden, D.B., Miller, J.R., and Simmons, G.A. (1992). Visual stimuli influencing orientation by larval gypsy moth *Lymantria dispar* (L.). *Canadian Entomologist*, **124**, 287–304.
133. Roessingh, P. and Städler, E. (1990). Foliar form, colour and surface characteristics influence oviposition behaviour in the cabbage root fly *Delia radicum. Entomologia Experimentalis et Applicata*, **57**, 93–100.
134. Rojas, J.C. (1999). Electrophysiological and behavioral responses of the cabbage moth to plant volatiles. *Journal of Chemical Ecology*, **25**, 1867–83.

135. Rojas, J.C. and Wyatt, T.D. (1999). Role of visual cues and interaction with host odour during the host-finding behaviour of the cabbage moth. *Entomologia Experimentalis et Applicata*, **91**, 59–65.
136. Rojas, J.C., Wyatt, T.D., and Birch, M.C. (2000). Flight and oviposition behavior toward different host plant species by the cabbage moth, *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae). *Journal of Insect Behavior*, **13**, 247–54.
137. Røstelien, T., Borg-Karlson, A.K., and Mustaparta, H. (2000). Selective receptor neurone responses to *E*-β-ocimene, β-myrcene, *E,E*-α-farnesene and *homo*-farnesene in the moth *Heliothis virescens*, identified by gas chromatography linked to electrophysiology. *Journal of Comparative Physiology A*, **186**, 833–47.
138. Röttger, U. (1979). Untersuchungen zur Wirtswahl der Rübenfliege *Pegomya betae* Curt. (Diptera, Anthomyidae). I. Olfaktorische Orientierung zur Wirtspflanze. *Zeitschrift für Angewandte Entomologie*, **87**, 337–48.
139. Rull, J. and Prokopy, R.J. (2000). Attraction of apple maggot flies, *Rhagoletis pomonella* (Diptera: Tephritidae) of different physiological states to odour-baited traps in the presence and absence of food. *Bulletin of Entomological Research*, **90**, 77–88.
140. Sadek, M.M., Hansson, B.S., Rospars, J.-P., and Anton, S. (2002). Glomerular representation of plant volatiles and sex pheromone components in the antennal lobe of the female *Spodoptera littoralis*. *Journal of Experimental Biology*, **205**, 1363–76.
141. Salama, H.S., Rizk, A.F., and Sharaby, A. (1984). Chemical stimuli in flowers and leaves of cotton that affect behaviour in the cotton moth, *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Entomologia Generalis*, **10**, 27–34.
142. Saxena, K.N. and Khattar, P. (1977). Orientation of *Papilio demoleus* larvae in relation to size, distance, and combination pattern of visual stimuli. *Journal of Insect Physiology*, **23**, 1421–8.
143. Scherer, C. and Kolb, G. (1987). Behavioral experiments on the visual processing of color stimuli in *Pieris brassicae* L. (Lepidoptera). *Journal of Comparative Physiology A*, **160**, 645–56.
144. Schneider, D. (1992). 100 years with pheromone research: an assay on Lepidoptera. *Naturwissenschaften*, **79**, 241–50.
145. Schöne, H. (1984). *Spatial orientation: the spatial control of behavior in animals and man*. Princeton University Press, Princeton, NJ.
146. Schoonhoven, L.M. (1987). What makes a caterpillar eat? The sensory code underlying feeding behavior. In *Perspectives in chemoreception and behavior* (ed. R.F. Chapman, E.A. Bernays, and J.G. Stoffolano), pp. 69–97. Springer, New York.
147. Shields, V.D.C. and Hildebrand, J.G. (1999). Fine structure of antennal sensilla of the female sphinx moth, *Manduca sexta* (Lepidoptera: Sphingidae). II. Auriculate, coeloconic, and styliform complex sensilla. *Canadian Journal of Zoology*, **77**, 302–13.
148. Shields, V.D.C. and Hildebrand, J.G. (2001). Responses of a population of antennal olfactory receptor cells in the female moth *Manduca sexta* to plant-associated volatile organic compounds. *Journal of Comparative Physiology A*, **186**, 1135–51.
149. Singer, M.C. (2000). Reducing ambiguity in describing plant-insect interactions: 'preference', 'acceptability' and 'electivity'. *Ecology Letters*, **3**, 159–62.
150. Singer, M.C., Stefanescu, C., and Pen, I. (2002). When random sampling does not work: standard design falsely indicates maladaptive host preferences in a butterfly. *Ecology Letters*, **5**, 1–6.
151. Skiri, H.T., Galizia, C.G., and Mustaparta, H. (2004). Representation of primary plant odorants in the antennal lobe of the moth *Heliothis virescens* using calcium imaging. *Chemical Senses*, **29**, 253–67.
152. Smith, B.H. and Getz, W.M. (1994). Nonpheromonal olfactory processing in insects. *Annual Review of Entomology*, **39**, 351–75.
153. Srinivasan, M.V., Poteser, M., and Kral, K. (1999). Motion detection in insect orientation and navigation. *Vision Research*, **39**, 2749–66.
154. Städler, E. (1992). Behavioral responses of insects to plant secondary compounds. In *Herbivores: their interactions with secondary plant metabolites*, Vol. II (2nd edn) (ed. G.A. Rosenthal and M.R. Berenbaum), pp. 45–87. Academic Press, New York.
155. Städler, E. (2002). Plant chemical cues important for egg deposition by herbivorous insects. In *Chemoecology of insect eggs and egg deposition* (ed. M. Hilker and T. Meiners), pp. 171–204. Blackwell, Berlin.
156. Städler, E. and Hanson, F.E. (1975). Olfactory capabilities of the 'gustatory' chemoreceptors of the tobacco hornworm larvae. *Journal of Comparative Physiology*, **104**, 97–102.
157. Steidle, J.L.M. and Van Loon, J.J.A. (2003). Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. *Entomologia Experimentalis et Applicata*, **108**, 133–48.
158. Steinbrecht, R.A. (1997). Pore structures in insect olfactory sensilla: a review of data and concepts. *International Journal of Insect Morphology and Embryology*, **26**, 229–45.
159. Stranden, M., Liblikas, I., Koenig,W.A., Almaas, T.J., Borg-Karlson, A.K., and Mustaparta, H. (2003).

(−)-Germacrene D receptor neurones in three species of heliothine moths: structure–activity relationships. *Journal of Comparative Physiology A*, **189**, 563–77.

160. Takabayashi, J., Dicke, M., and Posthumus, M.A. (1994). Volatile herbivore-induced terpenoids in plant–mite interactions: variation caused by biotic and abiotic factors. *Journal of Chemical Ecology*, **20**, 1329–54.
161. Thibout, E., Auger, J., and Lecomte, C. (1982). Host plant chemicals responsible for attraction and oviposition in *Acrolepiopsis assectella*. In *Proceedings of the 5th international symposium on insect–plant relationships*, Wageningen, 1982 (ed. J.H. Visser and A.K. Minks), pp. 107–15. Pudoc, Wageningen.
162. Thiéry, D. and Visser, J.H. (1986). Masking of host plant odour in the olfactory orientation of the Colorado potato beetle. *Entomologia Experimentalis et Applicata*, **41**, 165–172.
163. Thiéry, D. and Visser, J.H. (1987). Misleading the Colorado potato beetle with an odor blend. *Journal of Chemical Ecology*, **13**, 1139–46.
164. Tichenor, L.H. and Seigler, D.S. (1980). Electroantennogram and oviposition responses of *Manduca sexta* to volatile components of tobacco and tomato. *Journal of Insect Physiology*, **26**, 309–14.
165. Tingle, F.C., Heath, R.R., and Mitchell, E.R. (1989). Flight response of *Heliothis subflexa* (Gn.) females (Lepidoptera: Noctuidae) to an attractant from groundcherry, *Physalis angulata* L. *Journal of Chemical Ecology*, **15**, 221–31.
166. Tingle, F.C. and Mitchell, E.R. (1992). Attraction of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) to volatiles from extracts of cotton flowers. *Journal of Chemical Ecology*, **18**, 907–14.
167. Todd, J.L. and Baker, T.C. (1999). Function of peripheral olfactory organs. In *Insect olfaction* (ed. B.S. Hansson), pp. 67–97. Springer, Berlin.
168. Tommerås, B.A. and Mustaparta, H. (1987). Chemoreception of host volatiles in the bark beetle *Ips typographus*. *Journal of Comparative Physiology A*, **161**, 705–10.
169. Traynier, R.M.M. (1986). Visual learning in assays of sinigrin solution as an oviposition releaser for the cabbage butterfly, *Pieris rapae*. *Entomologia Experimentalis et Applicata*, **40**, 25–33.
170. Valterova, I., Bolgar, T.S., Kalinova, B., Kovalev, B.G., and Vrkoc, J. (1990). Host plant components from maize tassel and electroantennogramme responses of *Ostrinia nubilalis* to the identified compounds and their analogues. *Acta Entomologica Bohemoslovaca*, **87**, 435–44.
171. Van der Ent, L.J. and Visser, J.H. (1991). The visual world of the Colorado potato beetle. *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society*, **2**, 80–5.
172. Van der Pers, J.N.C. (1981). Comparison of electroantennogram response spectra to plant volatiles in seven species of *Yponomeuta* and in the tortricid *Adoxophyes orana*. *Entomologia Experimentalis et Applicata*, **30**, 181–92.
173. Van der Pers, J.N.C., Thomas, G., and Den Otter, C.J. (1980). Interactions between plant odors and pheromone reception in small ermine moths (Lepidoptera: Yponomeutidae). *Chemical Senses*, **5**, 367–71.
174. Van Loon, J.J.A., Everaarts, T.C., and Smallegange, R.C. (1992). Associative learning in host-finding by female *Pieris brassicae* butterflies: relearning preferences. In *Proceedings of the 8th International Symposium on Insect–Plant Relationships* (ed. S.B.J. Menken, J.H. Visser, and P. Harrewijn), pp. 162–4. Kluwer Academic Publishers, Dordrecht.
175. Vickers, N.J., Christensen, T.A., Baker, T.C., and Hildebrand, J.G. (2001). Odour-plume dynamics influence the brain's olfactory code. *Nature*, **410**, 466–70.
176. Visser, J.H. (1976). Electroantennogram responses of the Colorado beetle, *Leptinotarsa decemlineata*, to plant volatiles. *Entomologia Experimentalis et Applicata*, **25**, 86–97.
177. Visser, J.H. (1986). Host odor perception in phytophagous insects. *Annual Review of Entomology*, **31**, 121–44.
178. Visser, J.H. (1987). Cited in Ref. 146
179. Visser, J.H. (1988). Host-plant finding by insects: orientation, sensory input and search patterns. *Journal of Insect Physiology*, **34**, 259–68.
180. Visser, J.H. and De Jong, R. (1988). Olfactory coding in the perception of semiochemicals. *Journal of Chemical Ecology*, **14**, 2005–18.
181. Vité, J.P. and Gara, R.I. (1962). Volatile attractants from ponderosa pine attacked by bark beetles. (Coleoptera: Scolytidae). *Contributions of the Boyce Thompson Institute*, **21**, 251–73.
182. Vogt, R.G. (2003). Biochemical diversity of odor detection: OBPs, ODEs and SMNPs. In *Insect pheromone biochemistry and molecular biology* (ed. G.J. Blomquist and R.G. Vogt), pp. 391–445. Elsevier, Amsterdam.
183. Vosshall, L.B. (2003). Diversity and expression of odorant receptors in *Drosophila*. In *Insect pheromone biochemistry and molecular biology* (ed. G.J. Blomquist and R.G. Vogt), pp. 567–91. Elsevier, Amsterdam.
184. White, C. and Eigenbrode, S.D. (2000). Effects of surface wax variation in *Pisum sativum* on herbivorous

and entomophagous insects in the field. *Environmental Entomology*, **29**, 773–80.

185. Wibe, A. (2004). How the choice of method influence on the results in electrophysiological studies of insect olfaction. *Journal of Insect Physiology*, **50**, 497–503.
186. Wibe, A., Borg-Karlson, A.K., Norin, T., and Mustaparta, H. (1997). Identification of plant volatiles activating single receptor neurons in the pine weevil (*Hylobius abietis*). *Journal of Comparative Physiology A*, **180**, 585–95.
187. Willis, M.A. and Arbas, E.A. (1991). Odor-modulated upwind flight of the sphinx moth, *Manduca sexta* L. *Journal of Comparative Physiology A*, **169**, 427–40.
188. Zacharuk, R.Y. and Shields, V.D. (1991). Sensilla of immature insects. *Annual Review of Entomology*, **36**, 331–54.
189. Zohren, E. (1968). Laboruntersuchungen zu Massenanzucht, Lebensweise, Eiablage und Eiablageverhalten der Kohlfliege, *Chortophila brassicae* (Bouché) (Diptera: Anthomyiidae). *Zeitschrift für Angewandte Entomologie*, **62**, 139–88.
190. Zwiebel, L.J. (2003). The biochemistry of odour detection and its future prospects. In *Insect pheromone biochemistry and molecular biology* (ed. G.J. Blomquist and R.G. Vogt), pp. 371–90. Elsevier, Amsterdam.

CHAPTER 7

Host-plant selection: when to accept a plant

When engaged in host-plant finding, a herbivorous insect that touches a plant may enter what we will call the ‘contact phase’ of host-plant selection. This phase consists of a series of behavioural elements that serve to evaluate physical and chemical plant traits that could not be perceived from a distance.

7.1 The contact phase of host-plant selection: elaborate evaluation of plant traits

After initial plant contact, locomotion is often halted rather suddenly. This behaviour has been called arrestment; the insect tends to restrict its movements to a small area. For example, after a first brief landing an insect may fly off and immediately thereafter alight again on the same or a neighbouring leaf. A walking insect may start climbing along the plant stem and start moving in small circles over the plant surface. Caterpillars often sway their heads, probably facilitating orientation to odours. Plant structures such as leaf edges, veins, or stems seem to guide walking movements in this phase. During movement intermittent evaluation is performed, which shows itself as repetitive contacting of the plant surface with legs, antennae, mouthparts, or ovipositor; scratching and drumming with tarsi, antennating, palpating, and ovipositor-dragging are commonly observed types of behaviour. These movements are a direct response to physical and chemical contact cues offered by the plant. At the same time, volatile plant compounds that occur at relatively high concentrations in the leaf boundary layer can affect behaviour as well.[1,6,205] It is important to note that many species base their initial behavioural decision, either to proceed with evaluation or to reject the plant individual or organ just contacted, on physical and/or chemical surface characteristics.[11,59,207]

As a next step in the evaluation sequence, the insect may damage the plant and thereby release chemicals from the plant interior, comprising a complex mixture of primary and secondary metabolites. Injury is often inflicted by the insect's mouthparts and is designated as *test biting*, or *probing* in the case of piercing–sucking insects. A test bite is often smaller than a regular bite, and the plant material may be kept longer in the preoral cavity than during regular food intake. When the sensory information gathered during contact evaluation is judged positively by the central nervous system, acceptance, the final decision taken in the host-plant selection process, results and food intake or oviposition is started. The amount of sensory information gathered during the entire sequence has reached its maximum. Acceptance of food is normally expressed as a certain minimal bout of food intake. Acceptance of an oviposition substrate is evident from the deposition of one or more eggs. It should be noted that the actual amount of food intake or the number of eggs laid is highly variable and depends not only on the outcome of the sensory evaluation, but also on the physiological status of the individual (such as deprivation, egg load, age) and experience (see Chapter 8). From an evolutionary perspective, acceptance can be considered as the crucial decision taken during host-plant selection, as it has direct consequences for the acquisition of nutrients and energy or, in the case of oviposition, for the survival of progeny.

7.2 Physical plant features acting during contact

Upon contact with the plant an insect obtains additional information on plant quality that was not accessible during previous phases of host selection: tactile (mechanosensory) and contact-chemosensory (taste or gustatory) stimuli. Physical features of plant organs or tissues can profoundly influence host-plant selection behaviour. As discussed in Chapter 3, the presence of trichomes and wax crystal structures on the plant surface, leaf thickness and toughness, sclerotization, and high silica content may cause avoidance behaviour, and such plant traits are assumed to often fulfil a defensive function (Table 7.1).

Insects are equipped with numerous mechanosensory sensilla on all parts of their body,[120] and these probably code the relevant information on plant surface structure and texture. Taking plant features as a starting point, a few examples are presented in more detail to illustrate to what extent physical features of plants can affect host–plant selection. The primary interface in the contact phase of the insect–plant interaction is the plant surface: a plant does not suffer damage until the surface is penetrated, and we will examine its features first.

7.2.1 Trichomes

Plant surfaces are often covered with trichomes, which may be either glandular or non-glandular. These structures may hinder movement and feeding behaviour, especially of smaller insect and mite species. Intraspecific variation in trichome type or density has been successfully exploited in resistance breeding against some pest insects. In several cases the extent of pubescence is determined by one

Table 7.1 Selected examples of physical plant characteristics that affect host-plant selection by members of three insect orders: Lepidoptera, Hemiptera, and Coleoptera

	Plant species	Insect affected	Larva or Adult	Reference
Trichomes				
Non-glandular	Pigeonpea	African bollworm (*Helicoverpa armigera*) (Lep.)	L	173
	Cotton	Western lygus bug (*Lygus hesperis*) (Het.)	L + A	8
	Soybean	Bean leaf beetle (*Cerotoma trifurcata*) (Col.)	A	108
Glandular	Wild potato	Potato tuber moth (*Phthorimaea operculella*) (Lep.)	A	116
	Alfalfa	Potato leaf-hopper (*Empoasca fabae*) (Hom.)	L + A	160
	Datura wrightii	Tobacco flea beetle (*Epitrix hirtipennis*) (Col.)	A	78
Tissue thickness				
Pod	Soybean	Pod borer (*Grapholita glycinivorella*) (Lep.)	L	148
Stems	Tomato	Potato aphid (*Macrosipum euphorbiae*) (Hom.)	A	158
Leaf	Mustard	Mustard beetle (*Phaedon cochleariae*) (Col.)	L	214
Wax microstructure				
	Cabbage	Small cabbage white (*Pieris rapae*) (Lep.)	L	210
	Raspberry	Raspberry aphid (*Amphorophora rubi*) (Hom.)	A	113
	Mustard	Mustard beetle (*Phaedon cochleariae*) (Col.)	A	211

or two genes, which makes selection relatively easy.[152,200]

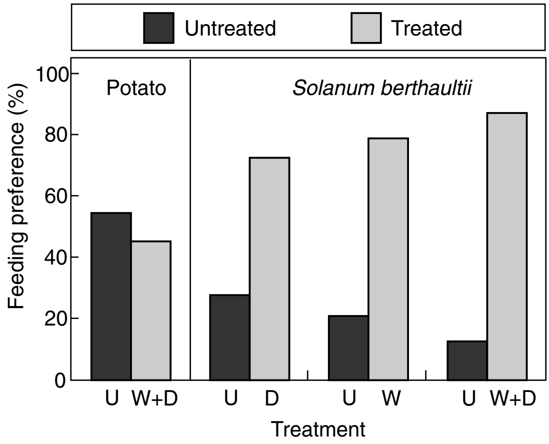


Figure 7.1 Effect of trichome removal of susceptible potato and resistant *Solanum berthaultii* by dipping (D, 95% ethanol dip), wiping (W, soft bristle-brush wipe), or combined dipping and wiping (W + D). Preference for treated versus untreated (U) leaves in adult Colorado potato beetles was determined in paired-choice experiments. It is seen that the combined wipe–dip treatment has no effect on potato, whereas all three treaments to remove trichome-produced substances from *S. berthaultii* result into a preference for the treated leaflets. (From Yencho and Tingey, 1994.)[237]

In glandular trichomes ('sticky hairs') we find a sophisticated combination of morphological and chemical plant resistance against insect colonization (see Section 4.7). The contents of glands associated with trichomes are liberated by mechanical damage caused by the moving insect, or are continuously exuding. Gland secretions may be repellent, deterrent, and/or toxic, or may effectively glue smaller species to the surface, after which they will succumb to starvation.[72] In larger species, active avoidance of plant species or cultivars carrying glandular trichomes on the basis of the allelochemicals they release has been demonstrated. A particularly well studied case is that of the Colorado potato beetle, which avoids the wild potato *Solanum berthaultii*. Adult beetles prefer to feed on the cultivated potato *Solanum tuberosum* in a choice situation, with *S. berthaultii* as the alternative. When *S. berthaultii* leaflets are appressed to *S. tuberosum* leaflets, these are avoided, indicating that deterrent chemicals are exuded from the trichomes of *S. berthaultii*. Removal of trichomes rendered *S. berthaultii* leaf material just as acceptable as *S. tuberosum* (Fig. 7.1).[72,237] When acetone leaf rinses of *S. berthaultii* were applied to *S. tuberosum* leaf discs, the non-volatile fraction was highly deterrent. Several different active compounds are involved, but their exact nature is as yet unknown. In the chrysomelid beetle *Gratiana spadicea*, a strict monophage on another *Solanum* species, isometric growth of the tarsungulus, a modified distal part of the tarsus, compared with allometric growth of other larval body features

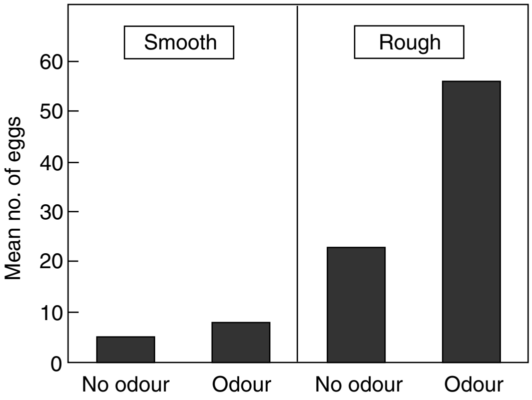


Figure 7.2 Effects of combinations of mechanosensory and olfactory cues on oviposition by the diamondback moth *Plutella xylostella*. Smooth or rough plastic caps were offered as an oviposition substrate, with or without 10 ppm allylisothiocyanate as the odour (this compound is a major volatile released by host plants of this Brassicaceae specialist). A rough surface baited with odour is by far the most stimulatory substrate; a rough substrate stimulates oviposition more strongly than a smooth substrate baited with odour. (From Gupta and Thorsteinson, 1960.)[75]

occurs as a morphological adaptation to attach to and move over the different trichome types on its host plant *S. sisymbriifolium*.[123]

7.2.2 Surface texture

Surface morphology may be quite important to female insects searching for an acceptable oviposition site. The diamondback moth *Plutella xylostella* prefers rough to smooth artificial surfaces (Fig. 7.2), and females deposit eggs mainly along leaf veins and small leaf and stem cavities. The cabbage root fly *Delia radicum* lays 2.5 times more eggs at the basis of artificial leaves with vertical folds compared with leaf models with horizontal folds. Moreover, the transition from leaf-blade exploration (see Fig. 6.1) to stem run is more likely to occur on leaves with vertical folds (Fig. 7.3).[165] The related anthomyid fly *Delia antiqua*, oligophagous on *Allium* spp., has been shown to take into account size, shape, and orientation of artificial plants. Integration of mainly mechanosensory information on these physical plant features enables the fly to select substrates that closely resemble its natural host plant. Numbers of eggs deposited at the basis of plant models are synergistically enhanced when a volatile characteristic of its host plants (dipropyldisulphide) is present.[80]

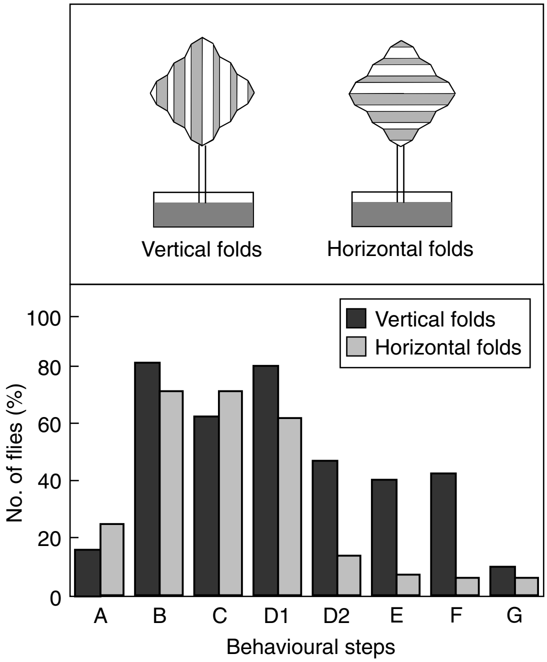


Figure 7.3 Influence of mechanosensory quality (horizontal versus vertical folds) of paper model leaves on oviposition behaviour of the cabbage root fly *Delia radicum*. For each behavioural element (A–G), the percentage of flies performing this step is displayed. A, short visit, no exploration of leaf; B, rest, grooming; C, leaf run with exploration of surface; D1, straight run on leaf borders or veins; D2, straight geotactic run on stem; E, horizontal circular run around stem, heading towards ground; F, walk from stem to ground, probing sand surface; G, oviposition attempts. Fewer flies complete the behavioural sequence on horizontally than on vertically folded surrogate leaves. The difference is associated with the transition from leaf exploration to stem run (D1 to D2), and significantly fewer females proceed to stem run (F) and oviposition (G). (From Roessingh and Städler, 1990.)[165]

7.3 Plant chemistry: contact-chemosensory evaluation

The previous sections clearly demonstrate that physical plant traits can affect host selection behaviour to an important extent. When we turn back to the high degree of host-plant specialization observed in herbivorous insects (see Chapter 2), it is evident, however, that the behavioural responses to physical plant features do not offer a satisfactory explanation for this taxonomic specialization. The main reason is that taxonomic patterns in physical and morphological features are absent,[93] which is in marked contrast with the taxonomic patterns observed in plant chemistry. Indeed, many plant families are characterized by secondary metabolites that do not occur in other families (see Chapter 4). Genera within plant families have also been found to contain either qualitatively specific or quantitatively dominant compounds that belong to the secondary chemistry characteristic of the family. Such chemotaxonomic patterns in the plant kingdom potentially provide a basis for host-plant specificity of herbivorous insects, and it is now firmly established that this potential has been utilized to an impressive degree of refinement.[11,180,203] We will expound on this paradigm in the rest of this chapter.

7.4 The importance of plant chemistry for host-plant selection: a historical intermezzo

The mechanism and function of the botanical specificity shown by most herbivorous insects has historically been a challenging phenomenon to biologists. It was about 200 years ago when the Swiss botanist A.P. de Candolle[41] implied that plant chemistry was the decisive factor in host-plant selection. J.H. Fabre[60] used the term 'botanical sense', referring to a sensory basis for behavioural specialization.[184] A tip of the veil over selection mechanisms was lifted by the Dutch botanist E. Verschaffelt,[231] who demonstrated that mustard oil glucosides (glucosinolates), which are taxonomically characteristic for cruciferous plants, are decisive factors for plant acceptance by caterpillars of the cabbage white butterflies *Pieris brassicae* and *P. rapae*.[185] The chemosensory basis of this behaviour was revealed only much later by the discovery of taste cells on the maxilla of the caterpillars that are specifically sensitive to these glucosides.[176] Dethier[47] demonstrated the role of terpenoids contained in essential oils of Apiaceae in host-plant acceptance of black swallowtail (*Papilio polyxenes*) caterpillars, specialized feeders on this plant family. Fraenkel,[63] in a seminal article entitled 'The raison d'être of secondary plant substances', brought together evidence that the food specificity of insects is based solely on the presence or absence of secondary metabolites and that several oligophagous species exploit taxon-specific secondary plant metabolites as recognition stimuli, whereas these compounds pose effective defensive barriers against non-adapted species. Dethier used the term 'token stimuli'* for the secondary plant substances that are employed as host-plant recognition signals by specialist herbivores.[48] Jermy has drawn attention to the role of deterrents, secondary plant substances inhibiting feeding or oviposition, and advocated the view that host-plant selection is based mainly on avoidance of deterrents present in non-hosts.[94,95,98] To counterbalance all attention paid to secondary plant compounds, Kennedy and Booth pointed to the combined importance of both secondary and primary plant metabolites in their 'dual discrimination' concept of host-plant selection.[103] These concepts have all contributed significantly to our current understanding of host-plant selection behaviour. They encompass the involvement of both primary and secondary compounds, and also their stimulatory and inhibitory effects on herbivore behaviour.

Below we will deal with the proximate mechanisms employed by plant-feeding insects in selecting plants primarily on the basis of their chemistry. In this chapter we focus on non-volatile (sapid) compounds that are perceived by gustatory receptors. A possible role of odours present at or near a feeding site has been much less studied, but there are indications that, during the contact phase, volatiles also may play a role.

Many plant chemicals are often confined to intracellular or extracellular compartments (see Section 4.11). An extracellular 'compartment' that is particularly relevant for each discussion of host-plant selection mechanisms is the plant cuticle. As mentioned before, chemicals present at the plant's surface may affect selection behaviour prior to any injury that would release cell contents, either as an innate response or as a result of experience.[36,106] Several groups of non-polar cuticular compounds, such as longer-chain alkanes and esters, probably occur only on the surface.[59,93] Sugars, amino acids,

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* Synonymous with 'sign stimuli', a term coined by E.S. Russell (Proc. Linn. Soc. London, 154, 195–216, 1943) in a paper probably unknown to Dethier.

and secondary metabolites, polar or non-polar, taxon-specific, or generally occurring, also occur on plant surfaces (Table 7.3). We indicate in the following discussion when behavioural responses have been found to surface-borne compounds.

7.5 Stimulation of feeding and oviposition

7.5.1 Primary plant metabolites

All plants contain carbohydrates and amino acids as primary metabolites resulting from their photosynthetic activity. There is ample evidence that most if not all herbivorous insects use carbohydrates, especially as feeding stimulants (Table 7.2). In most species studied, the disaccharide sucrose and its constituent monosaccharides fructose and glucose are the most powerful stimulants. These sugars are present at quite high concentrations (2–10% dry weight, which roughly corresponds to 10–50 mmol/l) in green leaves, and even higher in fruits and flower nectar (up to 0.25 mol/l). They generally stimulate feeding in a dose-dependent way (Fig. 7.4). Naturally they are also important nutrients needed to synthesize body tissue and to serve as energy sources (see Chapter 5).

Table 7.2 Comparative stimulatory effectiveness of various sugars for some herbivorous insects (for references see Bernays and Simpson (1982),[14] on which the table is based)

	Locusts		**Beetles**		**Caterpillars**	
	Locusta migratoria	***Schistocerca gregaria***	***Hypera postica***	***Leptinotarsa decemlineata***	***Pieris brassicae***	***Spodoptera* spp.**
Pentoses						
L-arabinose	+	•	•	–	–	–
L-rhamnose	–	–	•	•	•	–
D-ribose	–	–	•	–	–	•
D-xylose	–	–	•	–	–	–
Hexoses						
D-fructose	+++++	+++++	++++	+	–	+++++
D-galactose	++	+	•	+	–	++
D-glucose	+++	++++	+	+	++	++
D-mannose	–	+	++	–	–	+
L-sorbose	+	+	•	–	–	–
Disaccharides						
D-cellobiose	–	+	•	–	–	–
D-lactose	+	+	•	–	–	+
D-maltose	+++++	++++	++	–	–	+++
D-melibiose	+++	+++	•	–	–	++
D-sucrose	+++++	+++++	+++++	+++++	+++++	+++++
D-trehalose	+	++	+	+	–	–
Trisaccharides						
D-melizitose	++++	+	+++	++	–	++
D-raffinose	++++	+++	•	–	–	+++
Alcohols						
Inositol	+	•	•	–	–	–
Sorbitol	+	+	•	–	–	–
Mannitol	+	+	•	–	•	–

+++++, *highly stimulating;* +, *weakly stimulating;* –, *no effect;* •, *not tested.*

Reprinted from Bernays, E.A. and Simpson, S.J. (1982). Control of food intake. *Advances in Insect Physiology*, **16**, 59–118, by permission of the publisher, Academic Press Limited, London.

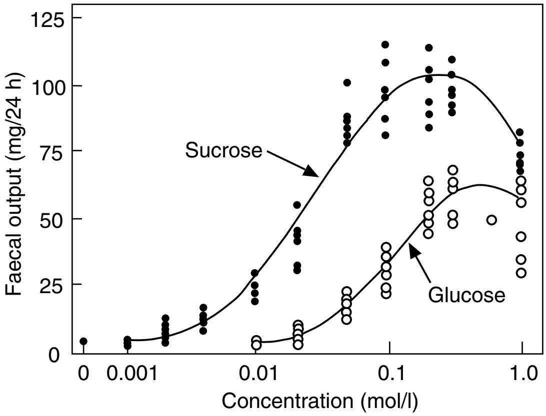

Figure 7.4 Behavioural response of *Pieris brassicae* larvae to two sugars, sucrose and glucose, incorporated in an agar-based gel medium (a mixture of agar, water, and cellulose). The parameter on the ordinate is dry weight of faecal output produced by six larvae over 24 h, a fair indicator of the amount of food intake. At lower sugar levels, sucrose is a considerably stronger feeding stimulant than glucose. (From Ma, 1972.)[114]

Although the protein content of plants is generally a limiting factor for the optimal growth of animals, protein molecules have not been found to stimulate feeding in herbivorous insects; however, it must be noted that few explicit attempts have been made to demonstrate this. In this context it is interesting to note that gustatory perception of a host-produced protein kairomone was recently demonstrated for a parasitoid wasp.[7] Whereas proteins do not seem to stimulate feeding behaviour directly, their building blocks, amino acids, act as feeding stimulants in several species.[14] However, the stimulatory action of the 20 naturally occurring amino acids may at the sensory level vary significantly between even closely related species.[190,227] Generally, 10 amino acids are nutritionally essential for insects, but these are not necessarily stronger stimulants than non-essential amino acids, nor stimulatory to more species.

Taste receptor cells for sugars and amino acids have been found in many species, and the ranking of chemosensory response intensities evoked by sugars or amino acids generally corresponds well with their behavioural effectiveness (but see Panzuto and Albert[153]) (see Section 7.8.5).[114,130] Although less well studied, other substances that take part in plant primary metabolism, such as the sugar alcohol inositol,[70] phospholipids, and nucleotides, and also minerals and vitamins (both nutritionally essential), are known to affect food acceptance in several species.[14,87]

Sugar and amino acid concentrations in different plant parts are spatially and temporally quite variable, variations that may be used as important cues for an insect when selecting a feeding site (see Chapter 4). The significance of sugars and amino acids as feeding stimulants can be quantified satisfactorily only by incorporation into a neutral substrate (such as an agar-based artificial substrate or filter paper), which in itself elicits little or no feeding and is devoid of deterrents. In this way their relative stimulatory effectiveness can be assessed. Such an approach has been carried out systematically for only few species.[87] In a no-choice situation, sucrose at the concentration levels that occur in plants may induce on its own a maximum feeding rate on artificial substrates without any further compounds added. However, how these rates relate to those achieved on plant tissues has not been directly compared, and they are therefore not directly indicative of the role of sugars in host selection behaviour. For example, oligophagous and polyphagous caterpillar species, even after being raised during four instars on an artificial medium, still preferred plant tissue when this was offered together with the diet in a dual-choice situation (J.J.A. van Loon, unpublished observations). Several problems arise when attempting to compare feeding stimulation by an intact plant with that offered by plant chemical constituents presented in an artificial diet. First, it is technically not possible to rule out differences in preference due to the obvious mechanosensory differences between the two. Second, in such studies artificial substrates generally contain a sugar and only one or two additional compounds, and are therefore nutritionally deficient. When feeding rate is measured indirectly by weight of faecal pellets or substrate consumed over several hours, each comparison with feeding rates on plant tissues is questionable, because feeding rate on a deficient diet may also be affected by positive physiological feedback resulting from low nutrient levels in the haemolymph (see Section 5.3.3).

Sugars have also been shown to promote oviposition in, for instance, the polyphagous European corn borer *Ostrinia nubilalis*.[44,46] Like most other ovipositing insects, the female moths do not seem to injure tissues and their oviposition response must be based on their perception of sugars present on the leaf surface. The dominant lipophilic consituents of leaf surfaces (alkanes, esters, fatty acids), to be considered as primary metabolites, are known to promote test-biting or probing, and subsequent feeding and oviposition in many insects, ranging from aphids to locusts (reviewed by Bernays and Chapman,[11] Eigenbrode,[58] and Eigenbrode and Espelie[59]).

Although primary plant substances, notably sugars and amino acids, do affect host-plant acceptance, the fact that they occur on the surface (Table 7.3) and in the interior of all plants, and that their concentrations vary greatly with plant developmental stage, age, physiological condition, and environmental factors, makes it unlikely that host-plant specificity can be explained by selection based solely on these categories of substances; in fact, no example is known. This notion leads us to consider the role of sapid plant secondary chemicals.

7.5.2 Plant secondary metabolites promoting acceptance: token stimuli

As noted in Chapter 4, plants offer a staggering diversity of secondary metabolites to herbivores. In this diversity taxonomic patterns are discernible: a chemically distinct group of substances often occurs in only one or a few related plant families. Some other categories of secondary metabolites, however, have a wide distribution among unrelated plant families, notably many phenolics and flavonoids.

The number of instances in which particular taxon-specific secondary metabolites act as feeding or oviposition stimulants to monophagous or oligophagous species has grown considerably since Verschaffelt's days.[231] Table 7.4 lists examples of feeding or oviposition activity governed by secondary plant substances in a number of food specialists belonging to different orders. In some cases the active compounds were found by means of an analogy approach (they had been found active to other insects feeding on the same plants); in other cases bioassay-guided fractionation (see

Table 7.3 Chemicals extracted and identified from leaf surfaces that have been found to affect insect behaviour

Chemical(s)	Plant species	Reference
Fructose, glucose, sucrose	Corn, sunflower	45
Amino acids	*Vicia faba, Beta vulgaris*	99
Amino acids	Corn, sunflower	45
Lipids	Cabbage and other species	59
Dulcitol (sugar alcohol) (**20**)	*Euonymus europaeus*	100
p-Hydroxybenzaldehyde	Sorghum	236
Glucobrassicin (glucosinolate) (**27**)	Cabbage	73, 228
Various glucosinolates	Oilseed rape	118
Phloridzin (phenolic) (**45**)	Apple	105
Anthraquinone (phenolic)	*Lolium perenne*	2
Luteolin, *trans*-chlorogenic acid (phenolics) (**36**)	Carrot	61
Falcarindiol (polyacetylene)	Carrot	206
Sesquiterpenes	Wild tomato	101
Triterpeneol acetate	Sweet potato	149
Duvane diterpenes, α- and β-diols, saturated hydrocarbons	Tobacco	92
Tyramine (alkaloid), *trans*-chlorogenic acid	*Pastinaca sativa*	33
Naringin, hesperidin (flavanones), quinic acid	*Citrus*	85
Aristolochic acids	*Aristolochia* spp.	147
Pyrrolizidine alkaloids	*Senecio jacobaea*	232
Various alcohols	*Populus*	110
α-Tocopherylquinone	*Populus*	110

Table 7.4 Monophagous and oligophagous herbivorous insects of different orders that use taxon-specific chemicals as token stimuli for host-plant acceptance, their host plant, the sign stimulus, and the chemical class to which it belongs; all cases where token-stimulus receptors have been identified are indicated

Insect species	Host plant	Sign stimulus	Chemical class	Reference	Receptor identified	Reference
Lepidoptera—feeding						
Pieris spp.	*Brassica* spp.	Sinigrin	Glucosinolates	231	Yes	176
Bombyx mori	*Morus* spp.	Morin	Flavonoid	178		
Euphydryas chalcedona	*Plantago*	Catalpol	Sesquiterpene	32		
Plutella xylostella	*Brassica* spp.	Sinigrin + flavonol triglucosides	Glucosinolate, Flavonoid	230	Yes	230
Tyria jacobaeae	*Senecio jacobaea*	Seneciphylline *N*-oxide	Pyrrolizidine alkaloid	20	Yes	20
Manduca sexta	*Solanum* spp.	Indioside D	Steroid glycoside	43	Yes	43
Lepidoptera—oviposition						
Pieris spp.	*Brassica* spp.	Glucobrassicin	Glucosinolate	164, 227	Yes	57, 209
Papilio polyxenes	*Daucus carota*	Luteolin-glycoside	Flavonoid	61	Yes	166
Battus philenor	*Aristolochia*	Aristolochic acid	Iridoid glycoside	175		
Junonia coenia	*Plantago*	Aucubin + catalpol	Iridoid glycoside, Sesquiterpene	154		
Eurytides marcellus	*Asimina triloba*	3-Caffeoyl-*muco*-quinic acid	Phenolic acid derivative	79		
Coleoptera—feeding						
Phyllotreta armoraciae	*Brassica* spp.	Sinigrin + flavonoid glycos.	Glucosinolate, Flavonoid	144		
Plagioderma versicolora	*Salix* spp.	Salicin	Phenolic	122		
Chrysolina brunsvicensis	*Hypericum*	Hypericin	Quinone	161	Yes	161
Diabrotica spp.	*Cucurbita* spp.	Cucurbitacins	Steroids (saponins)	128	Yes	141
Hymenoptera—oviposition						
Euura lasiolepis	*Salix* spp.	Tremulacin	Phenolic glycoside	171		
Diptera—oviposition						
Delia radicum	*Brassica* spp.	Glucobrassicin + 'CIF'	Glucosinolate	142	Yes	201
			Indole derivative	168	Yes	168
Psila rosae	*Daucus* spp.	Falcarindiol + bergapten, etc.	Polyacetylene Furanocoumarins	202	Yes	207
Delia antiqua	*Allium* spp.	*n*-Propyl disulphide	Disulphide	121	Yes	207
Mayetiola destructor	*Triticum aestivum*	Benzoxazolinone (MBOA) 1-Octacosanal	Hydroxamic acid Leaf wax aldehyde	139		
Homoptera—feeding						
Brevicoryne brassicae	*Brassica* spp.	Sinigrin	Glucosinolate	234		
Aphis pomi	*Malus*	Phloridzin	Chalcone	137		
Acyrthosiphon spartii	*Cytisus*	Sparteine	Alkaloid	199		
Megoura crassicauda	*Vicia* spp.	Acylated flavonol glycos.	Flavonoid	213		

glycos., glycoside; 'CIF', cabbage identification factor; MBOA, 6-Methoxybenzoxazolinone.

Appendix C) led to their identification. Especially for oviposition, the degree of stimulation by one or a few identified compounds was similar or nearly so to the response to total extracts of the host plant, or even to the intact host plant itself. These substances are good examples of 'token stimuli': their occurrence is restricted to certain plant taxa, and chemoreception of such compounds allows unambiguous recognition of the species' host plant. The best studied insect–plant interactions conforming to this principle are those between lepidopteran, dipteran, and coleopteran herbivores of Brassicaceae, Apiaceae, and Alliaceae.[203]

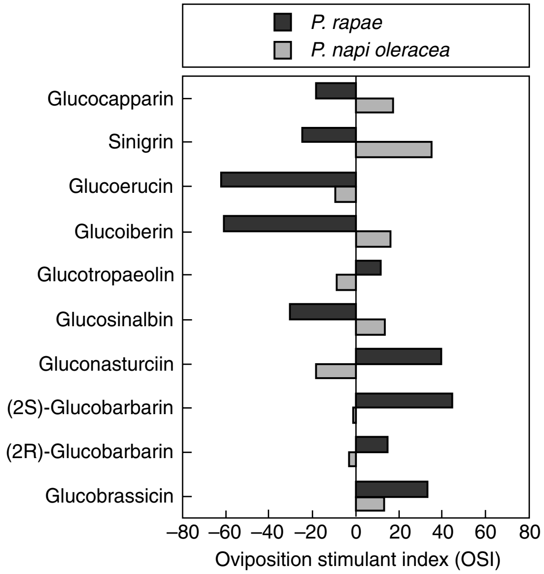

Figure 7.5 Stimulation of oviposition in *Pieris rapae* and *P. napi oleracea* by pure glucosinolates when sprayed on the non-host Lima bean (2 ml of a 0.1 mmol/l solution in water). The oviposition stimulant index (OSI) signifies the degree of preference in a dual-choice situation relative to a Lima bean plant that was sprayed with 2 ml of a 0.1-g leaf equivalent/ml cabbage extract. The major glucosinolate in the cabbage extract is glucobrassicin. A negative OSI means that the females preferred the cabbage extract-treated bean plant. Glucosinolates differ in their effectiveness to stimulate oviposition within each species, and both species differ in their preference hierarchy. (From Huang and Renwick, 1993.)[89]

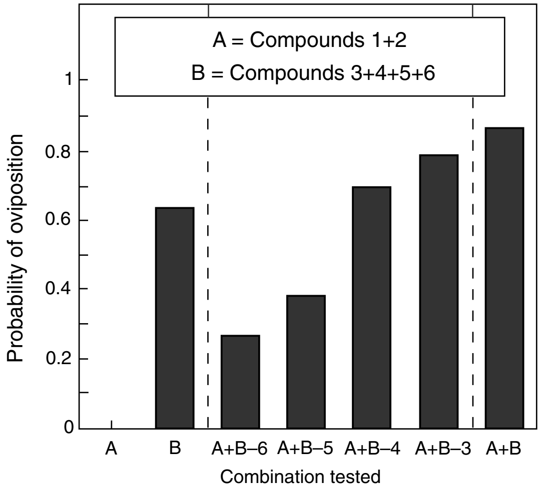

Figure 7.6 Probability of oviposition by individual females of *Papilio protenor* on filter-paper discs treated with different combinations of compounds isolated from the host plant *Citrus unshui*. Compounds tested were: (1) naringin 0.1%, (2) hesperidin 0.05%, (3) proline 0.2%, (4) synephrine 0.1%, (5) stachydrine 0.2%, and (6) quinic acid 0.2%. The mixture of compounds 1 and 2 (A) was inactive; the combination of A + B acted synergistically. Deletion of compound 4 (i.e. A + B − 4), 5, or 6 resulted in a significant reduction of stimulatory activity. (From Honda, 1990.)[84]

The complexity of the stimulatory chemical signal comprising secondary metabolites may differ considerably. In two species of cabbage white butterflies (*Pieris* spp.), a single glucosinolate isolated from the surface of cabbage leaves elicits a strong oviposition response when sprayed on artificial leaves or some non-host plants, such as *Phaseolus lunatus*.[164,228] Some other glucosinolates clearly differ in their stimulatory effect (Fig. 7.5). A much more complex situation has been revealed in swallowtail butterflies (*Papilio* spp.), where mixtures of compounds, only some specific to the host-plant taxon, were required to elicit a full behavioural responses (Fig. 7.6).[61,86,146]

Table 7.4 also demonstrates that, for different oligophagous species sharing the same host plants, the token stimuli may be qualitatively different. Examples of this are the carrot root fly (*Psila rosae*) and the black swallowtail (*Papilio polyxenes*), both living on carrot, the flea beetle *Phyllotreta armoraciae* and caterpillars of *Plutella* and *Pieris*, living on cabbage, and the leek moth (*Acrolepiopsis assectella*) and the onion fly (*Delia antiqua*), specialists of Alliaceae (reviewed by Städler[203]). When specific compounds have been shown to exert an appreciable stimulatory activity, as is the case for the examples cited above, often no further attempts have been made to identify additional compounds, despite the fact that the full behavioural response as occurs to intact plants was not obtained. An intriguing example is the cabbage root fly *Delia radicum*, for which glucosinolates act as taxon-specific oviposition stimulants;[167] these were assumed to be the prime phytochemicals on which host-plant specificity in this species was based. When a classical bioassay-guided isolation procedure was later carried out on leaf-surface extracts, a non-glucosinolate compound was quite unexpectedly found to be a much more powerful stimulant, evoking equal stimulation at 100 times lower concentrations than the most stimulatory glucosinolate (Fig. 7.7).[90,169]

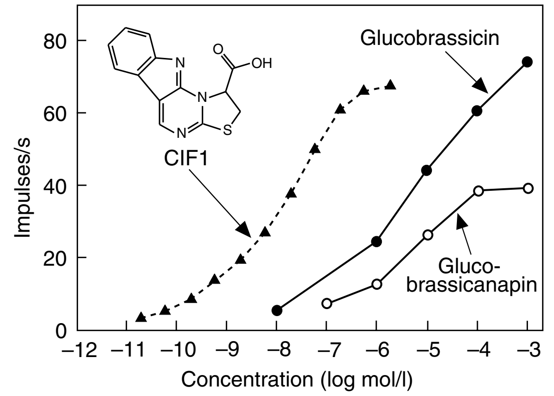

Figure 7.7 Dose–response curves of neural activity (number of action potentials in the first second after contact with sensillar tip) in taste hairs on the fifth tarsomere of cabbage root flies (*Delia radicum*) for the glucosinolates glucobrassicin and glucobrassicanapin, the strongest glucosinolate oviposition stimulants for this species, and for 'CIF1'. The latter chemical, which is found in surface extracts of cabbage leaves, is a much stronger oviposition stimulant than the two glucosinolates, but it does not belong to this chemical class and stimulates another cell than the glucosinolate-sensitive neuron. (Adapted from Roessingh *et al.*, 1992b;[168] and Hurter *et al.*, 1999.[90])

This compound, CIF, a thia-triaza-fluorene compound, stimulates another neuron in tarsal sensilla than the glucosinolate-sensitive neurons.[117]

For four decades two well studied specialists of solanaceous plants, the Colorado potato beetle *Leptinotarsa decemlineata* and the tobacco hawkmoth *Manduca sexta*, have defied the identification of secondary metabolites characteristic for Solanaceae acting as putative token stimuli.[97] Therefore, an alternative mechanism of host recognition in these species was proposed: that host plants are acceptable because they lack compounds that inhibit feeding (at least in any appreciable amount), whereas non-host plants are rejected because of the presence of deterrents.[95,97] Since these studies were performed, high-performance liquid chromatography, nuclear magnetic resonance spectroscopy, and mass spectrometry have undergone important innovations resulting in greatly improved sensitivity and precision. As a result of these technical advances, the long quest for token stimuli was able to be solved in both cases,[43,140] demonstrating the importance of tenacity in research. For the Colorado potato beetle, as yet unidentified minor steroidal alkaloids are implicated, whereas for the tobacco hawkmoth, a steroidal glycoside indioside D serves as token stimulus. Both types of compound were identified in potato plants. The number of insect–plant combinations that has been scrutinized in depth for the involvement of token stimuli is steadily growing (Table 7.4).

Especially in the case of surface-borne compounds (see Table 7.3), the concentration actually available to the gustatory sensilla when they contact an intact plant surface is unknown. Concentration values based on phytochemical extraction (assumed to be exhaustive) and quantities of surface-borne compounds can be expressed as micromoles per unit of surface area, but it is unclear which fraction of this quantity enters the taste sensilla and, consequently, what concentration is perceived. It is also remarkable that several insect species can be stimulated by polar chemicals present in the plant's epicuticle.[59] Possibly, taste sensilla possess as yet unknown mechanisms to release polar chemicals from the apolar waxy epicuticle, or they may penetrate the stomata to taste the leaf interior. It would be interesting to investigate these possibilities in more detail.

The solvent-based methods generally employed to extract them from the surface[111,207,232] have recently been disputed as being unsuitable to prove that chemicals are actually present in the epicuticular wax layer.[162]

7.5.3 Generally occurring secondary plant metabolites acting as stimulants

The number of insect species for which secondary plant metabolites found in unrelated plant families act as feeding stimulants is growing. This is particularly true for some phenolic acids and flavonoids (Table 7.5). For example, both caffeic acid (**8**) and its quinic acid ester chlorogenic acid (**11**) stimulate feeding in the silkworm *Bombyx mori*, oligophagous on Moraceae, whereas the latter compound also stimulates feeding in the Colorado potato beetle, specialized on some solanaceous plants.[87] Both the silkworm and the cotton boll weevil *Anthonomus grandis* are stimulated by the flavone-glycoside

Table 7.5 Flavonoids of different classes that have been implicated as insect feeding stimulants (modified from Harborne and Grayer, 1994)[77a]

Flavonoid class	Feeding stimulant flavonoid	Host plant and family	Insect species and (sub)order	Reference
Flavonol *O*-glycosides	Isoquercitrin, morin	*Morus alba* (Moraceae)	*Bombyx mori* (Lepidoptera)	77
	Isoquercitrin	*Gossypium hirsutum* (Malvaceae)	*Anthonomus grandis* (Coleoptera)	81
	Kaempferol 3-*O*-xylosylgalactoside	*Armoracia rusticana* (Brassicaceae)	*Phyllotreta armoraciae* (Coleoptera)	144
	Rutin	Many species	*Schistocerca americana* (Orthoptera)	16
	Rutin	Many species	*Helicoverpa zea* (Lepidoptera)	74
	Avicularin, hyperoside, rutin, quercitrin, isoquercitrin	*Fagopyrum esculentum* (Polygonaceae)	*Galerucella vittaticollis* (Coleoptera)	151
Flavone *O*-glycosides	7-α-L-rhamnosyl-6-methoxyluteolin	*Alternanthera phylloxeroide* (Amaranthaceae)	*Agasicles* sp. (Coleoptera)	238
	Luteolin-7-glucoside	*Salix* Salicaceae	*Lochmea capreae* (Coleoptera)	119
Flavone *C*-glycosides	Eight *C*-glycosylflavones	*Oryza sativa* (Poaceae)	*Nilaparvata lugens*, *Sogatella furcifera*, *Laodelphax striatellus* (Homoptera)	21
Dihydroflavonols and flavonone	Taxifolin, dihydrokaempferol, pinocembrin	*Prunus* spp. (Rosaceae)	*Scolytus mediterraneus* (Coleoptera)	109
Dihydrochalcone *O*-glycoside	Phloridzin (**45**)	*Malus* spp. (Rosaceae)	*Aphis pomi*, *Rhopalosiphum insertum* (Homoptera)	105
Flavanol *O*-glycoside	Catechin 7-*O*-xyloside	*Ulmus americanus* (Ulmaceae)	*Scolytus multistriatus* (Coleoptera)	55
Flavonoids	Isoorientin, tricin, tricin 7-*O*-glucoside	*Hyparrhenia hirta (Poaceae)*	*Locusta migratoria*, *Schistocerca gregaria* (Orthoptera)	31

isoquercitrin (quercetin-3-glucoside).[178] Polyphagous species also may be stimulated by the presence of flavonoids in their food. The ubiquitous quercetin glycoside rutin (**53**) has been documented as a feeding stimulant for both a locust (*Schistocerca americana*)[15] and *Helicoverpa virescens* caterpillars.[178] In view of the general occurrence of these secondary metabolites, the same reasoning applies as for nutrient chemicals: that it would be difficult to conceive how, for specialized species, these compounds could constitute an unambiguous signal for acceptance.

7.6 Inhibition of feeding and oviposition

Fraenkel[63] pointed out that secondary plant substances are defensive substances that inhibit food intake in the majority of plant-feeding insects, except for some specialized species, which may exploit these chemicals with a limited taxonomic occurrence as token stimuli enhancing acceptance. Relatively few studies have addressed rejection as a mechanism of host-plant specificity in a systematic way. Jermy clearly demonstrated that rejection of non-hosts by various insects is due to the presence of feeding inhibitors (feeding deterrents).[94,95] A 'sandwich' test was used in which a disc of the test plant species was offered between two discs of a host plant. This method allows exclusion of the absence of feeding stimulants as a cause of rejection or low preference of a non-host plant. Another detailed study was performed on two locust species, *Locusta migratoria*, a Poaceae specialist, and the polyphagous *Schistocerca gregaria*, and led to similar conclusions. Acceptance is one criterion for identifying host plants and non-hosts. Meal size is another, and this makes it possible to discern more grades of difference in the acceptability of plants. When meal size on a stimulatory artificial wheat flour substrate was used as a measure for acceptance, *Locusta* was seen to take full meals on (and thus fully to accept) Poaceae, but to take only small meals on non-hosts. All of the non-hosts contained deterrents, as did several less acceptable species of Poaceae. *Schistocerca*, on the other hand, showed much more variability in meal size. All plant species on which small meals were taken contained deterrents.[10]

7.6.1 Deterrency as a general principle in host-range determination

Comparative research on many herbivorous insects has uncovered several general principles underlying their responses to feeding deterrents. First, non-hosts commonly contain deterrents. Second, monophagous and oligophagous species are generally more sensitive to deterrents from non-hosts than polyphagous species (Table 7.6). This has been documented for locusts[10,11] and several caterpillar species.[28] Third, deterrents have been found not only in non-hosts, but in several instances also in acceptable plants, where their effect is apparently neutralized by the simultaneous presence of stimulants.[38,88,95] For several monophagous and oligophagous species for which token stimuli have been identified in their host plants, lack of stimulation together with possible deterrence offers an explanation for rejection of non-hosts, as infusion or coating with token stimuli renders some non-hosts acceptable and apparently overrides putative (weak) deterrents.[115,156,231]

A vast literature is available on the effects of many hundreds of secondary metabolites that inhibit insect feeding.[138] The accumulation of these data has been promoted by an interest in identifying plant-derived compounds with the prospect of their potential use in crop protection against insects (see Section 13.4).[65] Much less work has been done on oviposition deterrents,[163] but the information available suggests that, as in food-plant recognition, deterrence is in many insects an important mechanism in host-plant selection.

7.6.2 Host-marking as a mechanism to avoid herbivore competition

Gravid females in pursuit of an acceptable oviposition site are, after landing, influenced not only by the chemical make-up of the plant exterior but also by insect-produced compounds left by earlier visitors. Females of several butterfly, beetle, and fly species secrete, concomitantly with egg deposition, substances that inhibit the oviposition by conspecific females and inhibit the oviposition behaviour of females arriving later.[83,183] These substances have been termed 'host-marking pheromones' or 'epideictic pheromones'. From the few cases in which the chemical structure of such signal compounds has been elucidated, it appears that their chemical structures vary greatly.

Host-marking is a well known phenomenon in, for instance, many fruit flies. Female cherry fruit flies, *Rhagoletis cerasi*, drag their ovipositor over the fruit surface after an egg has been inserted under the skin of a cherry. During this dragging behaviour, marking substances are deposited on the fruit surface. Other females, after landing on an 'occupied' fruit, perceive these compounds with tarsal chemoreceptors. Investigations with synthetic analogues of the natural compound have shown that at the sensory level distinct structure–activity relationships exist,[208] suggesting that the marking pheromone stimulates a specialized receptor.

Table 7.6 Deterrent effects of compounds belonging to the major chemical classes of secondary plant substances to an oligophagous (O) lepidopteran and a polyphagous (P) lepidopteran or homopteran species

Compound	Chemical class	Insect species	Host-plant specificity	Effective concentration (ppm)	Inhibition (%)	Reference
Sinigrin (**61**)	Glucosinolate	*Papilio polyxenes*	O	900	66	29
		Mamestra configurata	P	3 100	50	192
Linamarin	Cyanogenic glycoside	*Heliothis subflexa*	O	1 235	40	18
		Heliothis virescens	P	12 350	40	18
Chlorogenic acid (**11**)	Phenolic acid	*Heliothis subflexa*	O	3 540	45	18
		Heliothis virescens	P	35 400	50	18
Phloridzin (**45**)	Flavonoid	*Schizaphis graminum*	O	200	50	56
		Myzus persicae	P	4 360	100	187
Strychnine (**65**)	Alkaloid	*Pieris brassicae*	O	30	100	114
		Mamestra brassicae	P	3 900	75	30
Caffeine (**9**)	Alkaloid	*Heliothis subflexa*	O	≤0.2	30	18
		Heliothis virescens	P	1	20	18
Ajugarin (**3**)	Diterpenoid	*Spodoptera exempta*	O	100	Thr	107
		Spodoptera littoralis	P	300	Thr	107
Azadirachtin (**6**)	Triterpenoid	*Pieris brassicae*	O	7	50	112
		Spodoptera frugiperda	P	315	50	159

Thr, threshold concentration.

In the case of two cabbage white butterflies (*Pieris brassicae* and *P. rapae*), egg washes were found strongly to deter oviposition, both intraspecifically and interspecifically. This indicates the involvement of a chemical marker substance that causes avoidance.[174] Some avenanthramide alkaloids isolated from the egg washes produced potent effects and were responsible for the activity of the crude egg wash. These compounds were found only in eggs of the genus *Pieris*, not in those from two other Pieridae nor in eggs from five non-pierid lepidopterans,[22] a specificity reminiscent of sex pheromones. *Pieris* butterflies do not exhibit dragging behaviour after egg deposition on the underside of a leaf. Leaves that carry egg batches are avoided for oviposition after landing on the upperside, and translocation of the identified putative marking substances was therefore investigated. Further studies could not demonstrate a translocation of the active principles of egg washes. Interestingly, however, fractions from surface extracts of leaves that had carried eggs were obtained that deterred oviposition but did not contain the egg-borne alkaloids.[23] In contrast to the cherry fruit fly, where the marking substance is produced solely by the insect, in the case of *Pieris* butterflies there is a role for the plant. Apparently, contact with *Pieris* eggs induces a change in the plant's surface chemistry and as yet unknown substances are produced that act as strong deterrents to ovipositing females. Since then, it has also been demonstrated in other insect–plant combinations that herbivore egg-deposition induces phytochemical responses in host plants that affect the behaviour of egg parasitoids.[82]

Several recent reviews have covered the behavioural and chemical ecology of oviposition-deterrent pheromones exhaustively.[3,150]

7.7 Plant acceptability: a balance between stimulation and deterrency

The stimulatory and inhibitory effects that plant chemicals, either primary or secondary, exert on the host-plant selection behaviour of herbivorous insects counteract one another and their balance determines the outcome of the decison-making process: rejection or variable degrees of acceptance, manifested as preference in choice situations.[11,51,129] When looking at the different categories of host-plant specialization, this 'balance model' is a useful concept in understanding selection behaviour. In polyphagous species, several ubiquitous primary metabolites suffice to stimulate feeding on many plant species and only those plants are rejected that produce deterrents of such a quality or in such a quantity that feeding stimulation is negated. A similar principle may govern host-plant range of those oligophagous species for which no taxon-specific token stimuli for host-plant recognition have been found (as discussed in Section 7.5.2). A third category includes oligophagous and monophagous species that do require token stimuli (see Table 7.4) for acceptance. For this category, the stimulatory signal is a taxon-specific secondary metabolite, often perceived by specialized taste receptors (see Section 7.8.4).

The view emerges that the mechanisms of host-plant selection employed in the different specialization categories are largely a matter of gradation rather than clearly definable and different modalities. In the third group, the association with a particular plant taxon has apparently given rise to a sensory specialization in the insect, constituting an overriding and unambiguous signal for recognition. It should be noted, however, that the balance between inhibitory and stimulatory chemicals is clearly asymmetrical. In other words, the effect of feeding inhibitors can be counterbalanced by feeding stimulants only to some degree. Above a certain level of inhibition no stimulants can evoke feeding. This is shown convincingly by sandwich tests, where the host-plant leaf discs do not neutralize the antifeeding effect of many or even most non-host-plant leaf discs.

7.8 Contact chemosensory basis of host-plant selection behaviour

7.8.1 Contact chemoreceptors

The behavioural responses to plant substances described above are based on the detection of these substances by gustatory neurons. Like olfactory cells, taste cells have their cell bodies located just below the cuticle and send a dendrite into a hair-, cone-, or papilla-like sensillum that has one terminal pore at its tip (see Fig. 6.14). Gustatory sensilla are located predominantly in the preoral cavity (e.g. the epipharyngeal sensilla) and on mouthparts, tarsi, ovipositor, and antennae (Fig. 7.8). Extremities equipped with sensilla can often be seen to move in such a way that the sensilla make brief intermittent contacts with the plant surface or plant cell contents during contact evaluation behaviour. The numbers of contact chemoreceptor sensilla differ markedly between species and between developmental stages within a species; in holometabolous insects especially, larvae have fewer than adults.[34] In grasshoppers, a trend is seen towards decreasing numbers of taste sensilla in more specialized feeders.[37] Monophagous acridids that feed on plants with high deterrent properties to other herbivores have the fewest sensilla.[24] In all cases, three to five taste neurons are typically associated with a taste sensillum, whereas most sensilla contain in addition a mechanoreceptive neuron (see Fig. 6.14).

7.8.2 Gustatory coding

Insect gustatory receptors are, like olfactory receptors (see Chapter 6), said to 'code' the complex chemistry of a plant by transducing the quality of the mixture of plant compounds into trains of action potentials (or 'spikes'), the electrical signal carrying neural information. The number of action potentials per unit of time and temporal details of spike trains, such as the distribution of intervals between spikes, contain information in an encoded form that travels without intermittent synapses to the first relay station, located in the suboesophageal or local segmental ganglion, a thoracic ganglion in the case of gustatory receptors on the leg, of the central nervous system.[104,134,170] The suboesophageal ganglion houses the motor neurons of the mandibular muscles that ultimately govern feeding activity.[25] Complex stimuli such as plant saps often evoke such trains in several cells innervating either the same sensillum or different sensilla simultaneously, and their axons converge in the segmental ganglia. Here integration occurs by merging with other incoming information from either peripheral receptors, such as mechanoreceptors, or internal receptors, and with input from other parts of the brain. After integration has taken place (a process that may take only a fraction

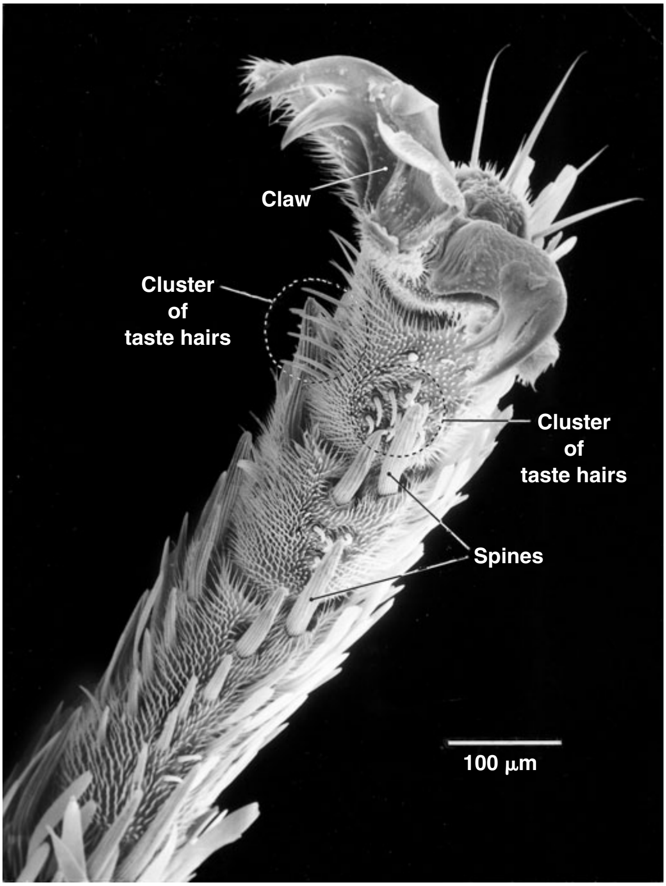

Figure 7.8 Scanning electron micrograph of the ventral side of the two distal tarsomeres of the prothoracic leg of a female *Pieris rapae* butterfly. Clusters of chemosensory hairs occur close to larger, non-innervated spines. (Reproduced by courtesy of E. Städler, Wädenswil, Switzerland).

of a second), feeding may or may not occur. A complicating factor is that the sensory message conveyed to the brain is by no means constant but varies with age, time of day, physiological state, and other biotic and abiotic parameters.[27] Compared with central processing of olfactory information (see Chapter 6), much less is known about central integration of contact-chemosensory information, despite its dominant role in host-plant selection.[170] Whereas olfactory information transmitted by receptors on antennae and mouthparts converge in glomeruli (well defined neuropils in the deuterocerebrum), information from the more widely dispersed gustatory receptors does not seem to converge in a specific area of the central nervous system.

One way to extract the sensory code is by analysing so-called 'input–output' relationships: the input (trains of action potentials) is quantified electrophysiologically by stimulating identified gustatory sensilla, and behaviour (the output) is quantified on the basis of either absolute amounts of food consumed or degree of preference for different feeding (or oviposition) substrates. On the basis of correlations between input and output, coding principles are inferred. In such studies, the sensillum rather than identified cells is often taken as the neurophysiological unit of response. This has a methodological rationale: in the extracellular recordings obtained by the standard tip-recording method, a separation of the extracellularly recorded spike trains arising from several taste neurons is technically difficult, even though computer-assisted spike-train analysis is available (see Appendix C). A second reason is that in only few cases has the specificity of neurons innervating a sensillum been analysed in sufficient detail to allow designation of a cell as, for example, a 'sugar-best', 'salt-best', or 'water' neuron.[50] Indeed, the study of the specificity spectrum or 'tuning' of cells is an enterprise in itself and has been carried out in relatively few cases for the eight-cell caterpillar taste system located in the maxillary taste hairs,[190] and to a limited extent for tarsal sensilla of *Pieris* butterflies[57,209] and *Delia* flies[167,196] in adult herbivorous insects. Most data are available for caterpillars and these show that remarkable differences in gustatory specificity exist even between closely related species.[190] Theoretically, there is no need to know these specificities in any detail in order to derive gustatory codes.[53] This notion defines the starting points of the two most frequently discussed concepts of chemosensory coding: labelled-line and across-fibre patterning, as discussed below.

7.8.3 Caterpillars as models for coding principles

Caterpillars, many species of which are very specialized feeders, have been favourite models for both sensory coding and behavioural studies. This is because several species were found in ablation studies to require only two maxillary hairs, each with four taste cells, for the integrity of host-plant discrimination behaviour (Fig. 7.9). The eight taste neurons represent about 10% of the total chemosensory complement (reviewed by Schoonhoven and van Loon[190]). One of the prime questions about chemosensory coding has been whether or not obvious differences exist between codes for the extreme decisions taken during selection behaviour: acceptance and rejection. Dethier's study on seven specialized caterpillar species (including both congeneric and unrelated species) led him to conclude that 'there is no universal difference between sensory patterns for acceptance and those for rejection'.[49] This suggests that the nervous system bases its decisions for behavioural output on the combined input from several taste neurons by reading synchronously across all afferent axons (fibres). This idea was formalized in the 'across-fibre' patterning concept of gustatory coding put forward in the vertebrate literature.[51] In an earlier study, the sensitivity spectra of the maxillary taste neurons of the seven species had been

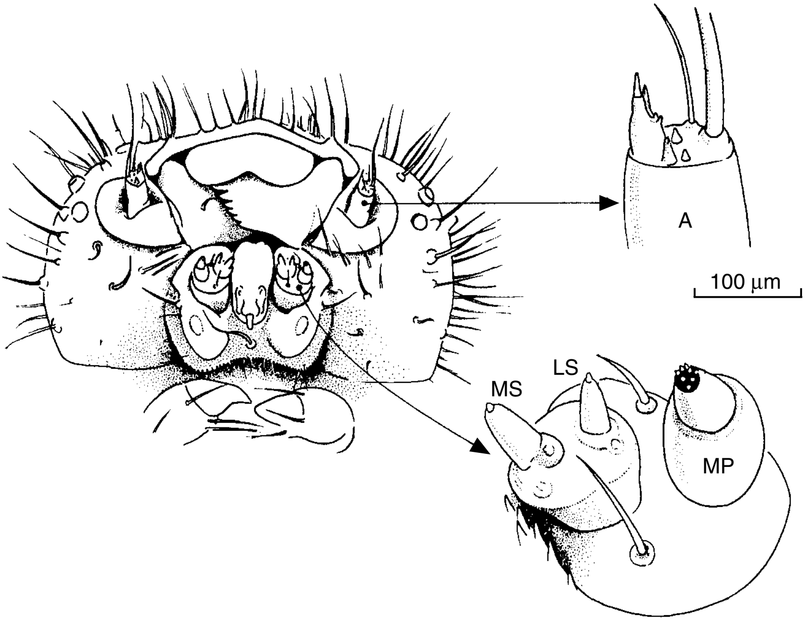

Figure 7.9 Diagram of the head of a caterpillar seen from below with enlargements of an antenna (A) and a maxilla. MP, maxillary palp; LS and MS, lateral and medial sensilla styloconica.

characterized to some extent and little evidence for specialized taste neurons had been found.[54] In both the oligophagous species *Manduca sexta* and polyphagous *Spodoptera* and *Helicoverpa* caterpillars, the ratio of firing between lateral and medial maxillary sensilla styloconica correlated with acceptability.[188,194] In *Manduca sexta*, across-fibre patterning has been proposed to function as the most probable mechanism of coding,[53,188] without detailed knowledge of gustatory cell specificities (see above). Evidently, it is the combined input from the two maxillary styloconic sensilla (and thus the across-fibre pattern generated by them) that determines the considerable subtlety in host-plant preference behaviour of these caterpillars.[182,194] A detailed study of coding of preference behaviour in *Manduca sexta* in response to three solanaceous plants pointed to the role of temporal patterning as another coding principle, which is superimposed on the across-fibre patterning. As a result of different adaptation rates of gustatory cells, the ratios of firing across different cells changes with time and therefore it is important to relate behavioural responses to the relevant time domain of the sensory response.[181]

Most investigations on chemosensory physiology and discrimination behaviour of caterpillars made in concert have focused on the eight taste neurons located on the maxillary galea. Additional taste organs are located in the preoral cavity. Many caterpillar species have two placoid sensilla on the epipharyngeal surface of the labrum. These sensilla have three chemoreceptor neurons each. Information from these sensilla may be involved in swallowing responses.[182] Colorado potato beetle adults and larvae also possess epipharyngeal sensilla,[127,131] whereas acridids have several groups on the epipharyngeal face of the labrum and on the hypopharynx.[34]

Recent studies suggested that input from epipharyngeal, antennal, and maxillary palp sensilla also contributes to food-plant discrimination.[40,69,230] Clearly, these organs merit more attention than they have received so far.

Adult insects have considerably more sensilla and taste neurons at their disposal than larvae.[34] This is especially true in the Lepidoptera and Coleoptera, in which the difference is at least 10-fold. Most probably these increased receptor numbers relate to the more complex behavioural tasks of adults. Whereas eating the right food is essential for larvae, adult insects represent the dispersal phase and must find, besides food, mating partners and, when female, oviposition sites. Despite the technical drawback of dealing with large receptor numbers, successful attempts have been made to analyse the coding of food preference in adult beetles[76,136] and moths.[26] By recording responses from a limited sample of the galeal sensilla of Colorado potato beetles (*Leptinotarsa decemlineata*) it appeared that saps from three host-plant species elicited a much more consistent response in the taste neurons than those from non-hosts. Preference among different solanaceous host plants is most probably based on neural messages coded in across-fibre patterns, but there are also indications for the use of labelled-line coding (Fig. 7.10).

7.8.4 Token stimulus receptors: unsurpassed specialists

An important event in the study of the chemosensory basis of host-plant specialization was the discovery of taste neurons that are highly sensitive to secondary plant substances in caterpillars of the large white butterfly (*Pieris brassicae*), a Brassicaceae specialist.[176] These cells are located in both sensilla styloconica on the galea of each maxilla and respond to a number of glucosinolates, which are characteristic of Brassicaceae. The two cells have overlapping, but not identical, sensitivity spectra. A certain minimal level of activity in these cells is required to signal acceptability of plant material. Such a chemoreceptor cell can be designated as a 'labelled line', that is, a line (axon) along which information is transferred to the brain that correlates quantitatively with the strength of the behavioural response. The influence of these labelled line-type receptors for token stimuli can be neutralized, however, by deterrents such as alkaloids or phenolic acids, which are perceived by so-called deterrent receptors.[186,224] A model for simple stimuli is given in Figure 7.11, but it is unknown whether this model also holds for natural (i.e. complex) stimuli, such as plant saps.[170]

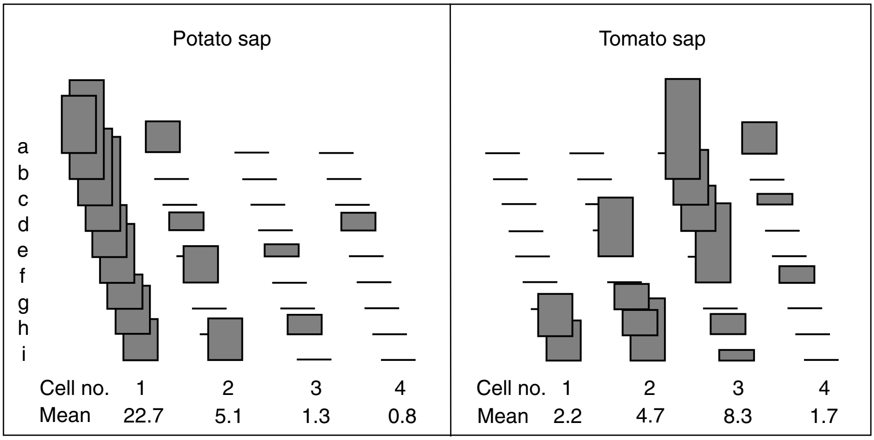

Figure 7.10 Across-fibre patterns of nine individual Colorado potato beetles (a–i) in response to leaf saps of potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*). The activity levels of four cells in taste sensilla on the galea of adults are represented as bars (mean values over nine individuals are indicated at the bottom). The main differences between the responses to potato and tomato are the low or absent activity of cell 1, together with higher activities of cells 2 and 3 in response to tomato sap, which provide the basis for behavioural discrimination between the two plants. (From Haley Sperling and Mitchell, 1991.)[76]

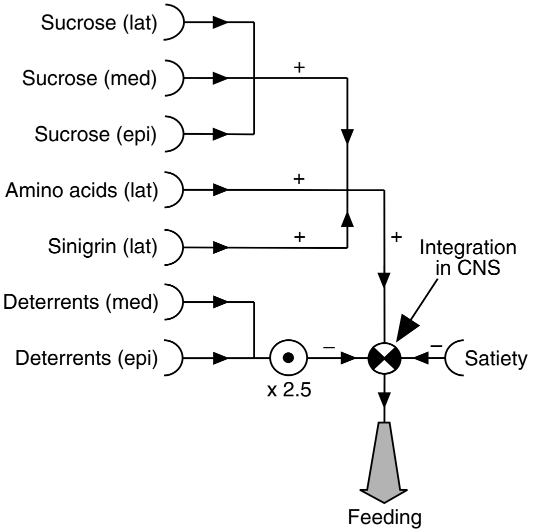

Figure 7.11 Schematic representation of how the inputs from different mouthpart chemoreceptors might be integrated within the central nervous system (CNS) to regulate feeding in the caterpillar of *Pieris brassicae*. Impulses from the sucrose, amino acid, and glucosinolate cells in the lateral (lat) and medial (med) sensilla styloconica on the galea and those from the epipharynx (epi) would have positive effects (+) tending to stimulate feeding, whereas inputs from the deterrent cells would have negative effects (−) tending to inhibit feeding. Satiety, representing a physiological parameter, would inhibit feeding when the gut is full. 'Feeding' or 'not-feeding' depends on the arimethric ratio between positive and negative inputs (i.e. nerve impulse frequencies). (From Schoonhoven, 1987.)[182]

Since then, more examples have been found of taste neurons that are specifically sensitive to a group of secondary plant metabolites. Such chemosensory cells seem to be quite typical for specialized herbivorous insects as they have not been documented for other animal groups, such as vertebrates, the taste system of which has been studied most extensively. This parallels the notion that the degree of host-plant specialization found in herbivorous insects is not equalled in other groups of herbivores, including vertebrates. In several monophagous or oligophagous species for which a token stimulus was identified through combined phytochemical and behavioural investigations, electrophysiological analyses revealed the presence of a corresponding token-stimulus receptor neuron. Stimulation of these cells is a signal to the brain: accept this food or oviposition site. For all cases documented so far such specialist cells detect stimulatory chemicals. This was also found for a maxillary taste neuron in the polyphagous caterpillar of *Estigmene acrea*, which displays an extreme sensitivity to the pyrrolizidine alkaloids that these caterpillars sequester for defence and pheromone production.[19] One case of a specialist deterrent neuron has been found (see below).[226]

It should be noted that the across-fibre patterns and labelled-line concepts are not mutually exclusive. The two concepts can be merged into one model in which across-fibre patterning (i.e. many cells, each with a different but overlapping sensitivity spectrum) participates in coding complex stimuli (such as plant saps). However, some cells with a narrow and well circumscribed sensitivity spectrum (labelled-line cells) may have a more pronounced or dominant influence, and may even play a decisive role in behavioural decisions. Likewise, deterrent cells may play a dominant or overriding role in the decision process. The presence of one or more dominant information channels does not rule out the function of the other taste neurons. The latter contribute to the decision process with more subtle details from the sensory evaluation of a plant's chemistry.

7.8.5 Sugar and amino acid receptors: detectors of nutrients

In Section 7.5.1 we discussed the general importance of primary metabolites as feeding stimulants. In caterpillars, some taste neurons sensitive to primary plant metabolites (e.g. sugars) that stimulate feeding are also specialized: they can be excited only by a narrow range of sugars, but not by, for example, amino acids or secondary plant metabolites.[190] In *Pieris* caterpillars, of the eight taste neurons present in the maxillary styloconic sensilla, two are 'sugar-best' cells with overlapping but different sensitivity spectra.[114] Stimulation of these cells is essential to induce adequate feeding rates. Amino acid-sensitive taste neurons have been found in various insect species (Table 7.7).

Sometimes perception of sugars and amino acids occurs via the same cell. In the adult Colorado potato beetle, a maxillary taste neuron sensitive to sugars also responds to two amino acids, gamma-amino butyric acid (GABA) and alanine, which are known to stimulate feeding.[133] Moreover, in larvae of the red turnip beetle (*Entomoscelis americana*) the sucrose-best cell responds to some sugars (e.g. sucrose and maltose) as well as to some amino acids,[132] whereas, curiously, in the adult insect this cell appears to be unresponsive to amino acids.[212] Clearly, the sensitivity spectra of taste neurons differ among species and may even vary between developmental stages of the same species. The most thoroughly investigated insect 'sugar-best' cells are those on the proboscis of several adult Diptera that are saprophagous. These cells generally combine sensitivity to sugars and amino acids, although separate receptor sites have been postulated.[141] In contrast, many (but not all)[17] Lepidoptera use separate cells to mediate information on the presence of sugars and amino acids.[190,227]

Another category of cell responding to generally occurring compounds is the 'inositol cell'. Several caterpillar species possess specialized receptor cells for sugar alcohols that stimulate feeding, such as inositol (**32**).[190] It is puzzling why most caterpillars tested have one, or often even two, of the eight maxillary chemoreceptor neurons specialized for inositol perception, because this seems a relatively high proportion of the available neuron population. Possibly inositol serves as a general indicator of plant quality, such as age and/or protein content.[143] In *Yponomeuta* species different taste neurons have been found for the two stereo-isomeric sugar alcohols dulcitol (**20**) and sorbitol (**64**), which constitute strong feeding stimulants to the caterpillars: a rosaceous non-host can be rendered acceptable to the celastraceous specialist *Yponomeuta cagnagellus* by impregnating *Prunus* foliage with dulcitol, the sugar alcohol that typically occurs at high concentrations in Celastraceae.[155]

7.8.6 Deterrent receptors: generalist taste neurons

In many caterpillar species one or more taste neurons have been identified that respond to a range of secondary plant substances occurring in non-host plants. These cells are designated 'deterrent receptors'. Treatment of otherwise perfectly acceptable host plants with such compounds, resulting in excitation of these deterrent receptor cells, leads to rejection of this plant material.[191] They can be considered to be generalist taste neurons in view of their sensitivity to a wide range of chemically unrelated classes of secondary plant compounds. The term 'generalist' does not mean, of course, that they respond to everything (e.g. sugars) or to all secondary plant compounds. For this cell type also, different caterpillar species display different sensitivity profiles.[181,190] How deterrent cells are able to

Table 7.7 Sensitivity spectra of amino acid receptors of the larvae of 12 lepidopterous species, one larval coleopteran, and one adult coleopteran

	Caterpillars												**Beetles**	
	P.b. **L**	**P.r.** **L**	**H.z.** **L**	**E.a.** **M**	**M.a.** **M**	**D.p.** **L**	**P.p.** **L**	**L.d.** **L**	**C.e.** **L**	**A.o.** **L**	**C.f.**[†] **L**	**G.g.** **L/M**	**L.d.**	**E.a.**
Reference	177, 227	54, 227	54	54	54	54	54	54	54	179	153	13	135	132
Arginine*	o	o	o	o	+	–	–	–	++		o	++		++
Histidine*	+++	+	o	o	o		o	o		+	+	+++		+
Isoleucine*	++	++	o	o	o	o	o	+		++		o	+	+
Leucine*	++	+++	++	+	o	o	o	o		+++	+	++	+	+
Lysine*	o	o										++		
Methionine*	++	+++	++	+	o	++	o	–	++	+	+	++		++
Phenylalanine*	+++	+	o	++	o	o	o	o		o	+	+	+	+
Threonine*	+	o	o	+	o	++	++	+		o		+	+	+
Tryptophan*	++	+	o	+	o	o	+	o		+		o		+
Valine*	++	++	–	+	++	++	o	o		++	+	+	+	
Alanine	++	++	o	+++	+++	++	o	o	–		+	+	+++	+++
Asparagine	++	++										+++		
Aspartic acid	o	o	o	o	+	o	o	o	++		+	+		++
Cysteine	+	o		++	o		–	o	++					
Cystine			++	o	+	+	o	o	+		+	+		
GABA	++	++										+	+++	+
Glutamic acid	o	o	o	++	++	o	–	o	o		+	++		++
Glycine	+	o	o	++	–	o	–	o			+	+	+	++
Proline	++	++	o	++	++	+++	o	o	++		o	+	++	++
Serine	++	++	o	+++	o	+++	++	o			+	+++	++	++
Tyrosine	o	o	+	+	o	o	o	o			+	o		

+++, Strong reaction; ++, medium reaction; +, mild reaction; o, no reaction; –, inhibition compared with control; L/M, Lateral/medial sensillum styloconicum; GABA, gamma-aminobutyric acid.

P.b., *Pieris brassicae*; P.r., *Pieris rapae*; H.z., *Helicoverpa zea*; E.a., *Ecrisia acrea*; M.a., *Malacosoma americana*; D.p., *Danaus plexippus*; P.p., *Papilio polyxenes*; L.d., *Lymantria dispar*, C.e., *Calpodes ethlius*, A.o., *Adoxophyes orana*; C.f., *Choristoneura fumiferana*; G.g., *Grammia geneura*; L.d., *Leptinotarsa decemlineata*; E.a., *Entomoscelis americana*.

* Essential amino acids.

[†] Different compounds were tested at different concentrations.

express this broad sensitivity is poorly understood but, on the basis of electrophysiological and genetic findings, there is evidence that different receptor sites tuned to, for instance, phenolic or alkaloid compounds, are involved.[71]

Pieris brassicae and *P. rapae* caterpillars have both a generalist and a more specialized deterrent cell in their maxillary taste hairs.[224] The specialist cell in the lateral sensillum (see Fig. 7.9) is a 'cardenolide-best' receptor by virtue of its extreme sensitivity to cardenolides (threshold about 10^{-8} mol/l). These compounds act as powerful steroidal deterrents and their presence in certain members of the insect's host-plant family, Brassicaceae, make these confamilial plant species unacceptable. The same cell also responds to phenolic acids and flavonoids, but only at a concentration more than 1000 times higher. The generalist deterrent neuron in the other hair, the medial sensillum, is also stimulated by cardenolides, but only at concentrations more than 10 times higher.[226] At present the cardenolide-sensitive cell is the only known example of a specialized deterrent cell. It can be envisaged to have evolved from a generalist deterrent cell by loss of receptor sites for other classes of deterrent such as alkaloids (Fig. 7.12).

Several recent studies have shown that so-called deterrent neurons in caterpillars act as 'labelled lines': the degree to which certain deterrent compounds coated on acceptable food causes rejection compared with untreated controls correlates nicely with firing rates of deterrent receptors in several caterpillar species (Fig. 7.13).[126,155,197]

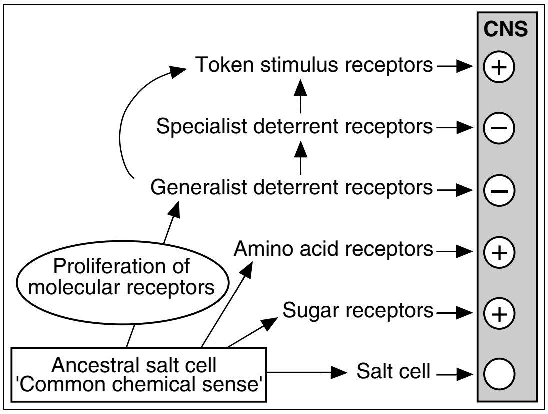

Figure 7.12 Hypothetical evolutionary pathways of gustatory receptor types in specialist herbivores. The circles containing plus and minus signs depicted in the central nervous system (CNS) represent excitatory and inhibitory synapses with the first-order interneurons. (From Schoonhoven and van Loon, 2002.)[190]

Above, we have tried to explain food-selection behaviour on the basis of knowledge of the stimulus spectra of the chemoreceptor neurons involved. Undoubtedly this deepened our insight into the plant cues responsible for the decision to feed or not to feed on a particular plant. It has also been argued, however, that gustatory neurons should be classified according to the behavioural effect of their activity rather than according to the type of chemical that causes their activity.[13] In this view, phagostimulatory and deterrent neurons are considered the basic labelled lines of the gustatory system.

7.8.7 Peripheral interactions

From the above discussions of both stimulant and deterrent receptors, a model emerges in which information on feeding stimulants and feeding deterrents is detected by independent chemoreceptor neurons and is transmitted separately to

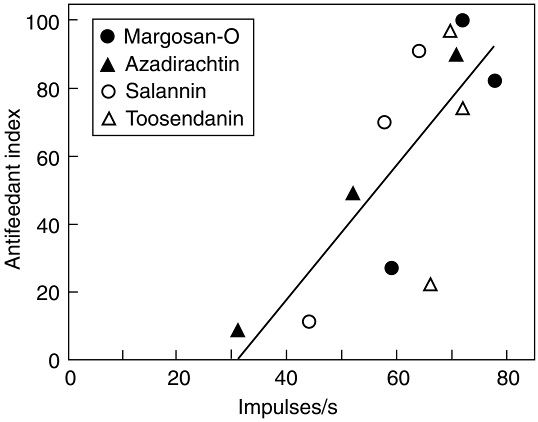

Figure 7.13 Relationship between antifeedant index (as determined by dual-choice tests) and spike frequencies of a deterrent receptor cell in the medial sensillum styloconicum of *Pieris brassicae* larvae. Impulse frequencies in response to three different concentrations of Margosan-O®, azadirachtin, salannin, and toosendanin have been plotted against antifeedant indices, at equimolar concentrations of the same compounds. A significant correlation is found between the intensity of the deterrent cell response and the antifeedant index. (From Luo *et al.*, 1995.)[112]

the brain; the subsequent weighing of inputs at the central level may conceivably occur according to arithmetical rules. Relatively simple arithmetical rules could be derived for *Pieris* and *Mamestra* caterpillars feeding on artificial diets.[186] Electrophysiological studies on other caterpillars, beetles, and grasshoppers revealed interactions in the chemosensory periphery that do not conform to linear arithmetic: the presentation of mixtures to a sensillum produces responses from one or several taste neurons that would not be expected from simple adding up of the responses to the individual components (Fig. 7.14). The effect of deterrent compounds on sugar-sensitive taste neurons has been well documented,[35,64,191] but species differ in terms of the extent to which the same compounds interact peripherally.[195] An example is the effect of an anthocyanin on the sugar-best cell in *Pieris* caterpillars. This flavonoid compound not only excites both the lateral and medial deterrent cell in galeal taste hairs but also inhibits the sucrose-sensitive cell present in both sensilla (Fig. 7.15). The reverse effect also occurs when stimulants suppress the response of deterrent receptors.[193]

Interactions at the sensory level are not necessarily inhibitory as in the examples discussed so far. They may also be of the synergistic type. For example, the sinigrin-sensitive cell in the polyphagous larva of *Isia isabella* is synergized by sucrose, which, when applied singly, stimulates only the sugar cell (Fig. 7.16).[54]

This differs from the case in which two compounds both stimulate the same cell but in combination evoke an increased reaction in comparison with the response to either compound alone. An example of the latter is known from the maxillary taste neurons of *Dendrolimus pini* caterpillars, which are responsive to a number of carbohydrates. When this neuron is stimulated by a mixture of glucose and inositol, a much stronger reaction is elicited than when either compound alone is applied.[182]

Peripheral interactions have been revealed in a growing number of cases since the attention has shifted from studying the stimulatory effects of pure compounds to the responses to binary mixtures of chemicals and to plant saps that represent natural but chemically undefined complex stimuli. Clearly, knowledge of responses to plant saps is important to the understanding of the chemosensory basis of selection among different host plants. Studying interactions in responses to binary mixtures may lead to results that are not representative of the complex stimulus situation of a leaf sap. The triterpenoid toosendanin is a powerful deterrent to *Pieris brassicae* larvae. It excites the medial deterrent neuron and inhibits sucrose and

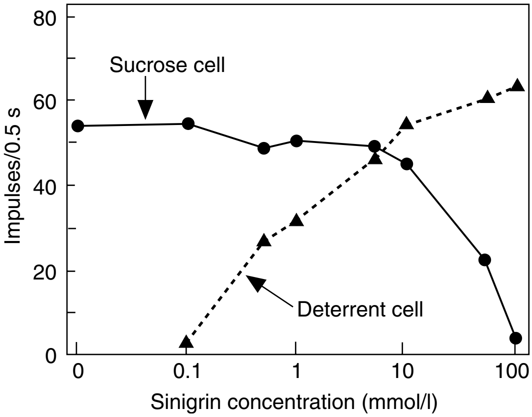

Figure 7.14 Impulse frequencies of the sucrose-sensitive and deterrent cells in the lateral sensillum styloconicum of *Heliothis subflexa* larvae upon stimulation with 5 mmol/l sucrose mixed with different concentrations of sinigrin. (Modified from Bernays and Chapman, 2000.)[12]

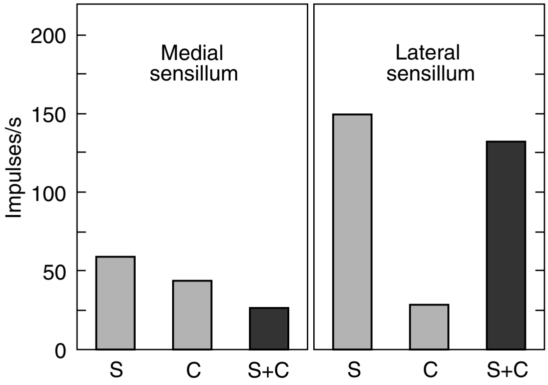

Figure 7.15 Inhibitory effects of cyanin chloride, an anthocyanin, on sugar responses in the two maxillary sensilla styloconica of *Pieris brassicae* larvae. Responses are presented as total impulse frequencies when stimulated with 15 mmol/l sucrose (S), 2.5 mmol/l cyanin chloride (C), and a mixture of these two stimuli (S + C). Neural activity in response to the mixtures is significantly lower in both sensilla than would be expected from adding up the values for single compounds. (From van Loon, 1990.)[224]

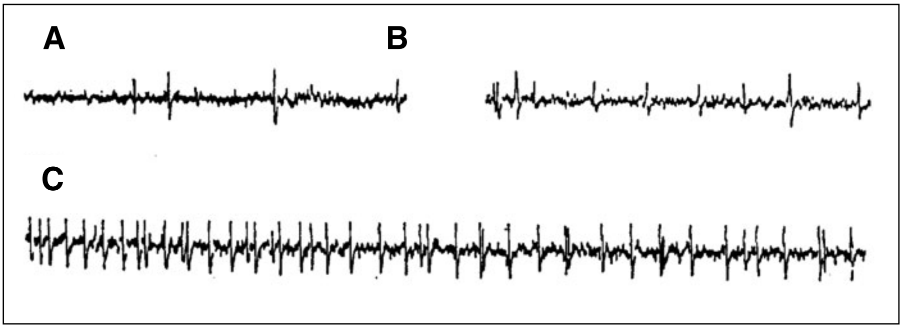

Figure 7.16 Synergistic receptor responses in the medial sensillum styloconicum on the maxilla of *Isia isabella* larvae. (**A**) Response to 0.001 mol/l sinigrin. (**B**) Response to 0.1 mol/l sucrose. (**C**) Response to a mixture of sinigrin and sucrose. The cell that responds preferentially to sinigrin alone shows a greatly increased response to the mixture. (From Dethier and Kuch, 1971.)[54]

glucosinolate neurons, both of which mediate feeding stimulation.[189] The triterpenoid azadirachtin also excites the medial deterrent cell, but to a lesser extent, and does not affect the responses of the stimulant receptor cells.[112] When the deterrent effects of toosendanin and azadirachtin are compared in a bioassay employing host-plant leaf discs, the response of the deterrent cell alone correlates well with the level of deterrent, and the putative contribution of the suppression of stimulant receptors by toosendanin seems to be minor if any. The occurrence and importance of peripheral interactions should therefore be studied by approaching the stimulus situation encountered during feeding or oviposition as closely as possible.[225]

It is unknown how peripheral interactions of different kinds arise. Probably, competitive or allosteric interactions occur at receptor sites in the membrane,[64,141] but as yet no direct proof for this is available. An additional mechanism for peripheral interactions may be electrotonic coupling between taste neurons, for which there is electrophysiological and ultrastructural evidence.[91,235]

When deterrent compounds affect stimulant receptors negatively, this of course contributes to the neural coding of deterrence. Additional mechanisms of deterrent coding are known, such as deterrents that produce irregular firing in sucrose-sensitive neurons. A systematic discussion of the various gustatory coding principles can be found in some recent reviews of this subject (Frazier,[64] Schoonhoven and van Loon,[190] Rogers and Newland[170]).

7.8.8 Host-plant selection by piercing–sucking insects

At this point it is important to be reminded of the two major feeding modes, biting–chewing and piercing–sucking, which present us with a dichotomy in the extent of our knowledge about the chemical cues involved. This is caused by the fact that piercing–sucking species are tissue and cell specialists. To identify the chemical cues they use in their selection of certain plant tissues or cells, chemical analysis of specific compartments is required; this is technically extremely difficult. As described in Chapter 3, in the Hemiptera, a prominent group of piercing–sucking insects, the mandibular and maxillary stylets are inserted into the subepidermal plant tissues. Different from mandibulate species that macerate entire tissues and rupture cells in the process, the hemipterans, especially some homopterans such as aphids, white flies, and other phloem-feeders, penetrate the plant tissues delicately with their stylets, seemingly to avoid cell damage altogether. The two maxillary stylets are interlocked in such a way that a double-barrelled tube is formed, one canal serving to imbibe food and the other to deliver saliva (see Fig. 3.2).

The stylets pierce the plant cuticle and then follow an intercellular route through the cell walls between mesophyll cells, heading towards vascular elements. Once inside the plant tissue, the stylets can be oriented into different directions in search of an acceptable feeding place (Fig. 7.17). The degree of control exercised over the stylets allows movements towards a vascular bundle, sometimes making 180° turns. Location of a phloem cell by using a chemical concentration gradient of sucrose or pH (both of which are higher in the phloem than in surrounding tissues) is still hypothetical. Stylet penetration behaviour of aphids, in particular, has been studied in detail using the electrical penetration graph (EPG) technique.[218] The stylets thus function as a self-penetrating electrode continuously monitoring the voltages at the stylet tip position in the plant. Different from the situation in biting–chewing species (see above), in aphids chemosensory evaluation of intracellular or extracellular contents of the leaf interior takes place only by internal chemoreception, in the epipharyngeal and hypopharyngeal taste organ, which contains about 100 taste neurons. The specificity and sensitivity of this chemosensory organ has defied electrophysiological approaches because of its minute size and anatomical position.

An EPG sequence can be characterized by three phases: a path phase, a xylem phase, and a phloem phase. The path phase, preceding a phloem or xylem phase, minimally lasts for about 10 min and reflects mechanical penetration through epidermis and other peripheral tissues as well as the excretion of saliva. Stylet penetration occurs in between the cells through the secondary cell wall and happens in a cyclical fashion of mechanical action and secretion of gelling saliva enveloping the stylets, called the salivary sheath. This salivary sheath is

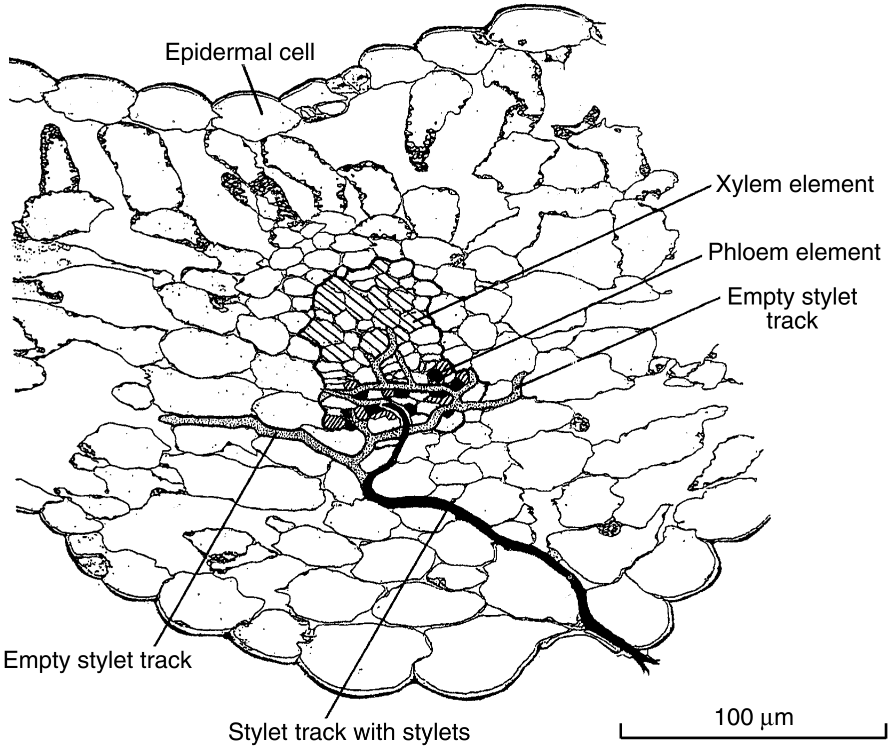

Figure 7.17 Stylet pathway of an aphid (*Aphis fabae*) feeding on a sieve element in the vein of a broad bean leaf. The stylet track shows many branches, representing earlier search movements during the process of phloem localization. The empty branches consist of salivary sheath material, which remains visible after the stylets have been withdrawn. (From Tjallingii and Hogen Esch, 1993, with permission.)[219]

left in the plant tissue and indicates where the stylet tips have been (Fig. 7.17). Brief cell punctures (lasting 5–10 s) along the pathway allow aphids (but not whiteflies) to sample cell contents, which are transported to the pharyngeal taste organ within a second, but the stylet pathway from cuticle to phloem remains largely extracellular.[219] When aphids are under water stress, a xylem phase can occur in the EPG, during which they imbibe water using an active muscle-driven sucking mechanism as the xylem is commonly under negative hydrostatic pressure. In the third phase the stylet tip reaches the target nutritional elements, the phloem cells. Two subphases occur, the first representing only the secretion of watery saliva, lasting for about a minute, followed or not by passive ingestion of phloem cell contents. Locating a suitable sieve tube to feed on is a tedious process and it seems that several phloem sieve cells are sampled prior to actual ingestion from one of them. The cues on which the selection of a particular phloem sieve element is based are unknown. On average, aphids commonly need between 2 and 7 h to initiate the first phloem phase, depending on the aphid–host plant combination.[220] Once accepted, they may tap a single sieve element continuously for several hours or days, sometimes up to 10 days.[217]

An important difference between aphids and other piercing–sucking insects on the one hand and biting–chewing species on the other is that, during penetration and ingestion, cells along the pathway to the target tissue are not damaged and contents of cytoplasm and vacuole do not mix. As many secondary plant substances in epidermal and mesophyll cells are stored in a glycosylated form and need first to be converted to the aglycone, which is the active defensive substance (see Section 4.11), piercing–sucking insects effectively circumvent this activation. However, aphid feeding results in large-scale transcriptome changes in plants. In a full-genome microarray study of *Arabidopsis*–attacker interactions, feeding by the aphid *Myzus persicae* resulted in the upregulation of about 830 genes—many more than the approximately 130 genes upregulated by the biting–chewing caterpillar *Pieris rapae*, or the 170 genes upregulated by *Frankliniella occidentalis*, a piercing species. It is interesting to note that concomitant feeding by *M. persicae* resulted in 1350 genes that were downregulated, whereas these numbers were only 60 for *P. rapae* and 30 for *F. occidentalis*.[42]

Owing to the fact that piercing–sucking species base their decisions to accept or reject a plant on mechanical and chemical cues that are located at the level of individual plant cell types, relatively little is known about the exact identity of these cues. Token stimuli seem to be involved in some cases, such as the aphid *Brevicoryne brassicae*, a specialist on Brassicaceae. In one of its host plants, *Sinapis alba* (white mustard), the dominant glucosinolate sinalbin was found to occur in much higher levels in epidermal cells of inflorescence stems than in leaf epidermal cells. *B. brassicae* greatly prefers to feed on the inflorescence stems than on leaves. EPG recording showed that, on leaves, many probes were made that lasted for less than 2 min, just long enough to penetrate the epidermis. In contrast, on inflorescence stems the very first probe in most cases lasted for much longer than 10 min and resulted in phloem feeding.[66] Rejection may be based on perception of allelochemicals occurring on the plant surface, perceived through antennal or tarsal contact chemoreceptors, in epidermal or mesophyll cells sampled during the pathway phase or based on substances occurring in phloem cells.[67,223] In only a few cases has the deterrent allelochemical been identified, for example DIMBOA (**17**), which occurs in maize and wheat, and is located mainly in the vascular bundle sheath cells but also at low concentrations in the phloem sap.[68]

7.8.9 Oviposition preference

Adult females, when accepting a plant to oviposit on, make a choice that is of crucial importance to the survival chances of their offspring, as the mobility and energy reserves of many first-instar larvae are so limited that their opportunities of finding a suitable host on their own are minimal. In two species of *Delia* flies (Diptera: Anthomyiidae), oligophagous on Brassicaceae, egg-laying is induced when the female contacts glucosinolates. Females show a distinct order of preference for different glucosinolates. The neural responses of glucosinolate-specific chemoreceptors located in sensory hairs on the tarsi, elicited by various glucosinolates, correlate well with behavioural responses to these compounds (Fig. 7.18). From these results, it is concluded that tarsal sensilla play an important, if not decisive, role in host-plant recognition.[204]

The two butterflies *Pieris rapae* and *P. napi oleracea* each display their own preference hierarchy for different glucosinolates (see Fig. 7.5). Electrophysiological studies on tarsal taste sensilla showed that, in these species too, the behaviourally most preferred compounds elicited the highest activity in glucosinolate-sensitive receptor cells.[209] Actually, it is surprising that such input–output relationships can be found, as the sample of sensory input quantified (the number of cells from which recordings were made relative to the total number of taste neurons present) comprises only 1–2% of the 2100 tarsal receptors available to the female. These findings, like those described above for caterpillars, indicate that the sensory characteristics vary among congeneric butterflies. Presumably the sensory system of each species is adapted to the host-plant selection typical of that particular species.

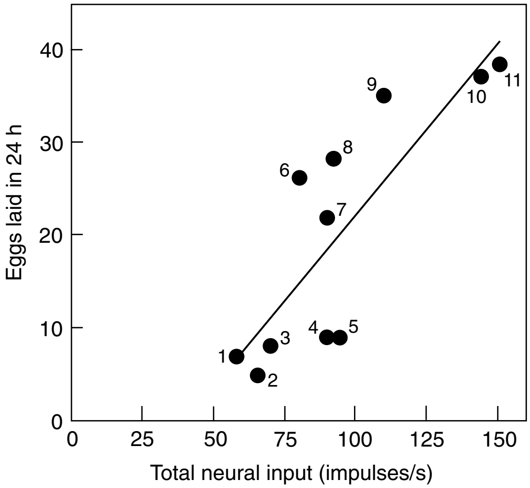

Figure 7.18 Relationship between summed neural input (impulses in the first second of stimulation) from two different receptor types on the legs and from labellar sensilla in the turnip root fly *Delia floralis* and oviposition behaviour (number of eggs laid over a 24-h period in a no-choice situation) for 11 different glucosinolates sprayed at 10^{-2} mol/l on an artificial leaf. A significant correlation was found between neural input and behavioural output. 1, Glucoerucin; 2, glucoiberin; 3, progoitrin; 4, sinalbin; 5, neoglucobrassicin; 6, sinigrin; 7, gluconapin; 8, glucotropaeolin; 9, gluconasturtiin; 10, glucobrassicanapin; 11, glucobrassicin. (From Simmonds *et al.*, 1994.)[196]

Even within a species (i.e. between subspecies), significant differences in sensory responses have been observed, indicating an evolutionary flexibility of the system. This is exemplified by two subspecies of *Pieris napi* that show consistent differences in their responses to glucosinolates (Fig. 7.19).[57] Cardenolides, deterrents to their larvae, have also proved to be powerful oviposition deterrents to adult females of both subspecies.[38] The cardenolides stimulate one cell, but do not affect the 'glucosinolate-best' cell. The preference hierarchy for glucosinolates is determined by the ensemble firing of the 'glucosinolate-best' neuron (positively correlated with higher preference) and the 'cardenolide-best' cell (negatively correlated with preference); the code is made up of a balance of two labelled lines, which is the most elementary across-fibre pattern. This example clearly shows the continuum that exists between the labelled-line and across-fibre pattern concepts. When a female alights upon a brassicaceous plant that carries a mixture of glucosinolates and cardenolides on its surface, both neurons are excited and the balance of activity between the two determines acceptance or rejection.

7.8.10 Host-plant selection: a three-tier system

Host-plant selection involves three major elements:

1. A peripheral chemoreceptive system, sensitive to multiple chemical stimuli, composed of phagostimulants and deterrents.

2. A central nervous system (CNS) tuned in such a way as to recognize sensory patterns. Certain patterns are recognized as acceptable, that is they release feeding or oviposition behaviour (which may be synergized by a 'motivation centre' (see Kennedy[102]); others promote rejection. The final decision is probably taken in the suboesophageal ganglion, but perhaps this process takes place at more than one location.[170] As a simplified model the 'lock and key' concept is a useful one. The sensory pattern of a specialist feeder would, in this

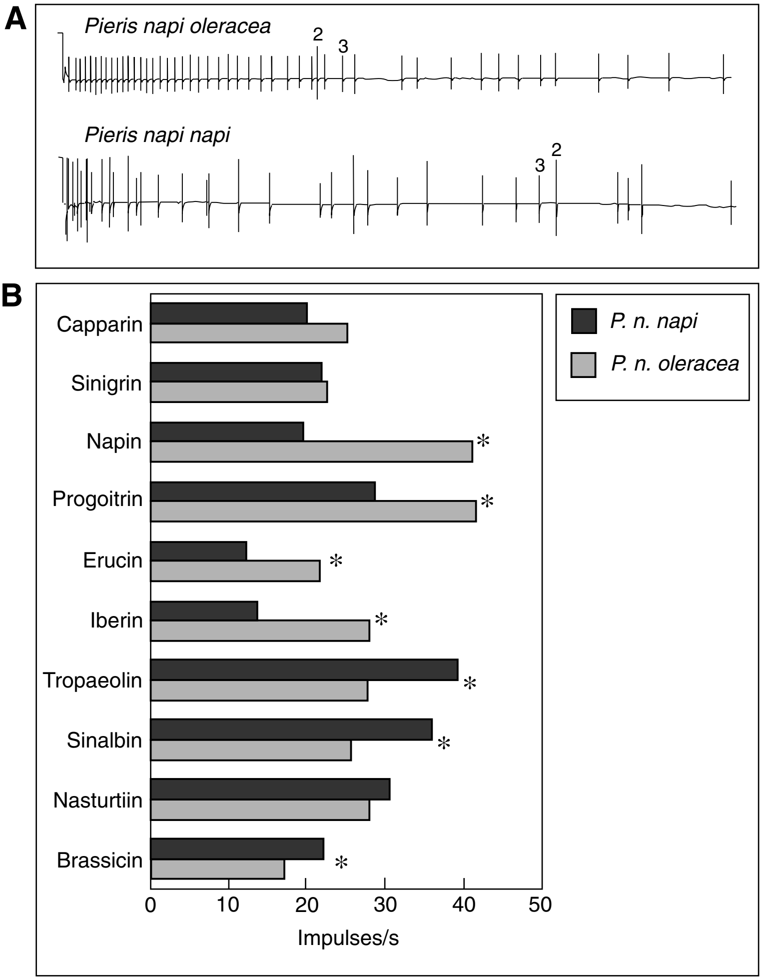

Figure 7.19 (**A**) Recordings of electrophysiological activity from taste hairs on tarsi of female *Pieris napi oleracea* and *P. napi napi* in reponse to the glucosinolate gluconapin at 10 μg/ml. In *P. napi napi* a second cell (designated as '2') fires much more frequently than in *P. napi oleracea*. (**B**) Response profiles to 10 different glucosinolates (the response strength is expressed as the number of one spike type [indicated by '3' in (**A**)] in the first second of stimulation); significant differences were found between both subspecies for seven compounds (indicated by *). (From Du *et al*., 1995.)[57]

model, have to match more closely a certain norm set by the CNS, in order to trigger feeding activity, than is the case for food generalists. In other words, many different receptor activity profiles or 'keys' fit into the CNS template ('lock') and release feeding in generalists, whereas the 'locks' of specialists are more selective (Fig. 7.20).[182]

3. A third component determining acceptance or rejectance of a potential food plant, involving the contribution of an internal chemosensitive system. This system warns the CNS when food composition differs too much from physiological requirements, resulting in a change of food selection (see Section 5.3.3).

Of course, the three-tier system of host-plant selection, with its interacting elements of receptors, CNS, and nutritional feedback, is not a closed system but perpetually interacts with numerous ecological constraints.[184]

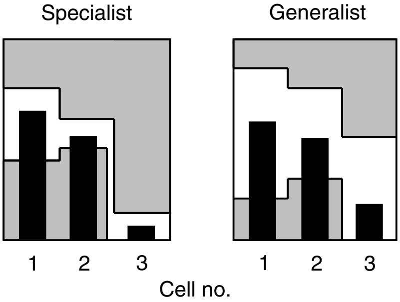

Figure 7.20 Model of CNS processing of sensory input in a food specialist and a generalist. The black bars represent action potential frequencies in three chemoreceptors (1–3) when stimulated by an acceptable food plant. The white space of the 'lock' reflects the variation permitted to the sensory input while still being interpreted as acceptable. Cell 3 is a deterrent receptor. (From Schoonhoven, 1987.)[182]

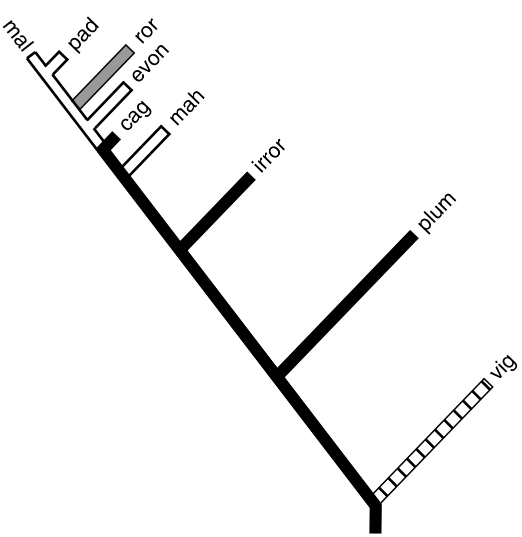

Figure 7.21 Phylogenetic tree of the nine west European *Yponomeuta* species based on allozyme data and the botanical status of their host plants. *Yponomeuta* species: cag, *cagnagellus*; evon, *evonymellus*; irror, *irrorellus*; mah, *mahalebellus*; mal, *malinellus*; pad, *padellus*; plum, *plumbellus*; ror, *rorellus*; vig, *vigintipunctatus*. Host-plant affiliations: black, Celastraceae; white, Rosaceaea; shaded, Salicaceae; black and white, *Y. vigintipunctatus* feeds on Crassulaceae, but its sister species, *Y. yamagawanus*, feeds on *Euonymus* (Celastraceae). (Redrawn from Menken *et al.*, 1992.)[125]

7.9 Evolution of the chemosensory system and host-plant preferences

In the foregoing sections we expanded on the crucial importance of the chemosensory system in host-plant acceptance or rejection behaviour. Combined with the basic observation (see Chapter 2) that specialists greatly outnumber generalists, several authors have drawn attention to the hypothesis that the evolution of insect–plant relationships depends upon evolutionary changes in the insect nervous system, at both peripheral and central levels.[9,96] In this scenario, the chemosensory system is supposed to change first, before any host-plant shift or preference change that might result into new insect–plant associations. Selection is subsequent to the genetic changes in the insect's plant-recognition system, because the origination of a new genome that codes for new plant preferences will be successful only if it is able to tolerate the many selective factors of physiological (plant toxins; see Sections 5.4 and 11.7) and ecological nature (e.g. natural enemies; see Section 11.7) to which it will be exposed.[9] Constraints on the evolution of the insect's nervous system would predominantly, but not necessarily, result in the emergence of new specialists from specialists.

This scenario implicitly touches on the genetic basis of chemoreceptor specificity in herbivorous insects. The smaller the number of genes that are involved in determining host-plant specificity and preference, the more likely it is that these traits can evolve rapidly. According to crossing experiments with *Papilio* butterflies, changes at relatively few genetic loci could have large effects on the host-preference hierarchy of these butterflies.[215] Studies on the function and genetics of insect chemoreceptors suggest that a single mutation could change monophagy to polyphagy, and vice versa.[52] A study on interspecific hybrids of two *Yponomeuta* species provided evidence that sensitivity to a feeding deterrent, a chalcone glycoside, is inherited via a single dominant gene.[222] Host-plant shifts based on reduced sensitivity to deterrents has possibly been an important factor in the evolution of *Yponomeuta* (Table 7.8).[124] Phylogenetic reconstruction of this genus suggests that Celastraceae comprise the ancestral host-plant family and that a shift occurred to Rosaceae (Fig. 7.21). One species, *Yponomeuta malinellus*, feeding on the rosaceous genus *Malus* and a second species, *Y. rorellus*, that

Table 7.8 Chemosensory sensitivities in galeal styloconic taste receptors in four *Yponomeuta* species (Yponomeutidae), specialized feeders associated with host plants that are chemotaxonomically unrelated (data from van Drongelen, 1979)[221]

Species	**Host plant (family)**	**Taste receptor specificities in lateral/medial sensilla styloconica**			
		Dulcitol	**Sorbitol**	**Phloridzin**	**Salicin**
Yponomeuta cagnagellus	*Euonymus europaeus* (Celastraceae)	+/+ *	–/–	–/+	n.t./+
Yponomeuta padellus	*Prunus/Crataegus* spp. (Rosaceae)	±/–†	+/–*	–/+	+/+
Yponomeuta malinellus	*Malus* spp. (Rosaceae)	–/–	+/–	–/–*	+/+
Yponomeuta rorellus	*Salix* spp. (Salicaceae)	–/–	–/–	–/+	–/+ *

+, Receptor sensitive; –, receptor insensitive; n.t., not tested.

* Compound present in host plant mentioned.

† Dulcitol is present in some rosaceous host plants in low concentrations (about 10% of the levels found in Celastraceae).

made a shift to yet another plant family, the Salicaceae, both lack sensitivity at the chemoreceptor level to compounds found specifically in *Malus* and *Salix*, respectively, whereas these substances act as deterrents to the other species studied (Table 7.8). The converse process may also occur, leading to a narrowing of host range. It is also possible that the diet breadth of a monophagous species becomes wider when deterrent neurons lack sensitivity to certain classes of deterrent substances. This appears to be the case for some mutants of the silkworm *Bombyx mori*, that will feed on some food plants that are normally rejected.[5] A better characterization of the number and specificity of receptor sites is needed to support such scenarios.

If a gene that encoded a deterrent receptor molecule were to be expressed in a taste neuron sensitive to stimulants such as sugars, this would explain how token-stimulus receptors originated (see Fig. 7.12). Indeed, that this can occur has been found in a taste mutant of *Drosophila melanogaster*.[4] Genomic analysis of *Drosophila* has uncovered a family of 60 genes that code for seven-transmembrane proteins that are candidate taste receptor proteins.[39] Study of the ligand specificity of these receptor proteins and homologues in herbivorous insects has high potential to increase our insight into taste-mediated host-plant recognition and its evolution. Apart from different receptor sites, different intracellular transduction mechanisms allow sensory discrimination of different classes of deterrents. The tobacco hornworm *M. sexta* can discriminate salicin from aristolochic acid because different transduction pathways operating in the same deterrent neuron are involved.[71]

Specialists equipped with chemoreceptors that recognize taxonomically specific plant chemicals as token stimuli thus appear to use an unambiguous signal offering a high degree of contrast with the multitude of competing signals. This system evidently presents fitness advantages. Genetically determined changes in host-plant preferences can occur in only seven generations,[198] and switches to closely or distantly related host plant species and broadening or narrowing of host-plant specificity have been reported (see Chapter 11). Such behavioural changes might originate from mutational changes in receptor protein genes that are assumed to occur stochastically. Whether such mutations are spreading depends on the selective advantages they confer and on genomic and developmental constraints.[96]

7.10 Conclusions

Once an insect has established contact with a potential host plant, elaborate evaluation behaviour ensues during which the insect uses both mechanosensory and chemosensory (predominantly taste) stimuli offered by the plant. Host-plant selection is to a large extent governed by a central neural evaluation of the profiles of chemosensory activity generated by the multitude of taste stimuli presented by the plant. Our current knowledge of these responses suffers from a bias towards water-soluble compounds, and virtually nothing is known about gustatory perception of the apolar phytochemicals that dominate leaf surfaces.

The chemical quality of the plant as perceived by the insect is encoded in the combined activity of taste neurons that have different degrees of specificity, ranging from highly specialized (e.g. token-stimulus receptors) to generalized (e.g. deterrent receptors). At the behavioural level it has been amply documented that acceptance is determined by the balance between stimulatory and inhibitory compounds. Only recently has it been demonstrated that this balance can be traced, partially at least, to activity at the chemosensory level as the ratio of identifiable stimulatory and inhibitory inputs. This ratio often seems to determine preference hierarchies in a straightforward way. In other cases, however, the codes have not been cracked and it is clear that uncovering the physiological basis of the often intricate discriminatory ability of plant-feeding insects is a continuing challenge. Because more and more peripheral interactions are being found in response to mixtures, the study of chemosensory activity profiles in response to plant saps, the natural stimuli, is implicated as the best way to account for the possibly large numbers of interactions occurring under field conditions.

Clearly our still limited knowledge of insect taste receptors permits the conclusion that herbivorous insects possess a highly sensitive system that allows them to detect subtle chemical differences between plants and between plant parts. Another important conclusion is that each species, perhaps even each biotype, is equipped with a species-specific sensory system that is optimally equipped to discriminate between host plants and non-hosts, as well as among different hosts.

The existence of highly specialized taste receptors in several specialized feeders, together with evidence for the existence of several receptor sites with monogenic inheritance on generalist deterrent neurons, is relevant to understanding the evolution of specialization and the probability of host shifts. As the activity of such receptors is the basis of acceptance or rejection decisions, mutational changes at the receptor level will affect the insect's behaviour. When, for instance, the sensitivity to a (class of) deterrent(s) is lost by a mutation in the respective receptor site, a host shift may occur (see Fig. 7.12). Previously unacceptable plants containing such deterrents may then become acceptable and the host range is expanded when the deterrents involved are not lethally toxic (and many of them are not). Support for this scenario comes from the lepidopterous genus *Yponomeuta*.

The evolution of food-plant specialization so characteristic for herbivorous insects may thus be determined to a considerable degree by neural constraints, at either the sensory or the central level.[225]

7.11 References

1. Ahmad, S. (1982). Host location by the Japanese beetle: evidence for a key role for olfaction in a highly polyphagous insect. *Journal of Experimental Zoology*, **220**, 117–20.
2. Allebone, J.E., Hamilton, R.J., Bryce, T.A., and Kelly, W. (1971). Anthraquinone in plant surface waxes. *Experientia*, **27**, 13–14.
3. Anderson, P. (2002). Oviposition pheromones in herbivorous and carnivorous insects. In *Chemoecology of insect eggs and egg deposition* (ed. M. Hilker and T. Meiners), pp. 235–63. Blackwell, Berlin.
4. Arora, K., Rodrigues, V., Joshi, S., Shanbhag, S., and Siddiqi, O. (1987). A gene affecting the specificity of the chemosensory neurons of *Drosophila. Nature*, **330**, 62–3.
5. Asaoka, K. (2000). Deficiency of gustatory sensitivity to some deterrent compounds in 'polyphagous' mutant strains of the silkworm, *Bombyx mori. Journal of Comparative Physiology A*, **186**, 1011–18.
6. Baur, R., Feeny, P., and Städler, E. (1993). Oviposition stimulants for the black swallowtail butterfly: identification of electrophysiologically active compounds in carrot volatiles. *Journal of Chemical Ecology*, **19**, 919–37.
7. Benedet, F., Leroy, T., Gauthier, N., Thibaudeau, C., Thibout, E., and Renault, S. (2002). Gustatory sensilla sensitive to protein kairomones trigger host acceptance by an endoparasitoid. *Proceedings of the Royal Society, Biological Sciences, Series B*, **269**, 1879–86.
8. Benedict, J.H., Leigh, T.F., and Hyer, A.H. (1983). *Lygus hesperus* (Heteroptera: Miridae) oviposition behavior, growth and survival in relation to cotton trichome density. *Environmental Entomology*, **12**, 331–5.
9. Bernays, E.A. (2001). Neural limitations in phytophagous insects: implications for diet breadth and

evolution of host affiliation. *Annual Review of Entomology*, **46**, 703–27.

10. Bernays, E.A. and Chapman, R.F. (1978). Plant chemistry and acridoid feeding behaviour. In *Biochemical aspects of plant and animal coevolution* (ed. J.B. Harborne), pp. 91–141. Academic Press, London.
11. Bernays, E.A. and Chapman, R.F. (1994). *Host-plant selection behaviour of phytophagous insects*. Chapman & Hall, New York.
12. Bernays, E.A. and Chapman, R.F. (2000). A neurophysiological study of sensitivity to a feeding deterrent in two sister species of *Heliothis* with different diet breadths. *Journal of Insect Physiology*, **46**, 905–12.
13. Bernays, E.A. and Chapman, R.F. (2001). Taste cell responses in the polyphagous arctiid, *Grammia geneura*: towards a general pattern for caterpillars. *Journal of Insect Physiology*, **47**, 1029–43.
14. Bernays, E.A. and Simpson, S.J. (1982). Control of food intake. *Advances in Insect Physiology*, **16**, 59–118.
15. Bernays, E.A., Howard, J.J., Champagne, D., and Estesen, B.J. (1991). Rutin: a phagostimulant for the polyphagous acridid *Schistocerca americana. Entomologia Experimentalis et Applicata*, **60**, 19–28.
16. Bernays, E.A., Howard, J.J., Champagne, D., and Estesen, B.J. (1991). Rutin: a phagostimulant for the polyphagous acridid *Schistocerca americana. Entomologia Experimentalis et Applicata*, **60**, 19–28.
17. Bernays, E.A., Chapman, R.F., and Singer, M.S. (2000). Sensitivity to chemically diverse phagostimulants in a single gustatory neuron of a polyphagous caterpillar. *Journal of Comparative Physiology A*, **186**, 13–19.
18. Bernays, E.A., Oppenheim, S., Chapman, R.F., Kwon, H., and Gould, F. (2000). Taste sensitivity of insect herbivores to deterrents is greater in specialists than in generalists: a behavioral test of the hypothesis with two closely related caterpillars. *Journal of Chemical Ecology*, **26**, 547–63.
19. Bernays, E.A., Chapman, R.F., and Hartmann, T. (2002). A highly sensitive taste receptor cell for pyrrolizidine alkaloids in the lateral galeal sensillum of a polyphagous caterpillar, *Estigmene acraea. Journal of Comparative Physiology A*, **188**, 715–23.
20. Bernays, E.A., Hartmann, T., and Chapman, R.F. (2004). Gustatory responsiveness to pyrrolizidine alkaloids in the *Senecio* specialist, *Tyria jacobaeae* (Lepidoptera, Arctiidae). *Physiological Entomology*, **29**, 67–72.
21. Besson, E., Dellamonica, G., Chopin, J., Markham K.R., Kim, M. Koh, H.S., *et al.* (1985). *C*-Glycosylflavones from *Oryza sativa. Phytochemistry*, **24**, 1061–4.
22. Blaakmeer, A., Stork, A., Van Veldhuizen, A., Van Beek, T.A., De Groot, Æ., van Loon, J.J.A., *et al.* (1994a). Isolation, identification, and synthesis of miriamides, new hostmarkers from eggs of *Pieris brassicae. Journal of Natural Products*, **57**, 90–9.
23. Blaakmeer, A., Hagenbeek, D., Van Beek, T.A., De Groot, Æ., Schoonhoven, L.M., and van Loon, J.J.A. (1994b). Plant response to eggs *vs.* host marking pheromone as factors inhibiting oviposition by *Pieris brassicae. Journal of Chemical Ecology*, **20**, 1657–65.
24. Bland, R.G. (1989). Antennal sensilla of Acrididae (Orthoptera) in relation to subfamily and food preference. *Annals of the Entomological Society of America*, **82**, 368–84.
25. Blaney, W.M. and Simmonds, M.S.J. (1987). Control of mouthparts by the subesophageal ganglion. In *Arthropod brain* (ed. A.P. Gupta), pp. 303–22. Wiley, New York.
26. Blaney, W.M. and Simmonds, M.S.J. (1990). A behavioural and electrophysiological study of the role of tarsal chemoreceptors in feeding by adults of *Spodoptera, Heliothis virescens* and *Helicoverpa armigera. Journal of Insect Physiology*, **36**, 743–56.
27. Blaney, W.M., Schoonhoven, L.M., and Simmonds, M.S.J. (1986). Sensitivity variations in insect chemoreceptors; a review. *Experientia*, **42**, 13–19.
28. Blaney, W.M., Simmonds, M.S.J., Ley, S.V., and Katz, R.B. (1987). An electrophysiological and behavioural study of insect antifeedant properties of natural and synthetic drimane-related compounds. *Physiological Entomology*, **12**, 281–91.
29. Blau, P.A., Feeny, P., and Contardo, L. (1978). Allylglucosinolate and herbivorous caterpillars: a contrast in toxicity and tolerance. *Science*, **200**, 1296–8.
30. Blom, F. (1978). Sensory activity and food intake: a study of input–output relationships in two phytophagous insects. *Netherlands Journal of Zoology*, **28**, 277–340.
31. Bouaziz, M., Simmonds, M.S.J., Grayer, R.J., Kite, G.C., and Damak, M. (2001). Flavonoids from *Hyparrhenia hirta* Stapf (Poaceae) growing in Tunisia. *Biochemical Systematics and Ecology*, **29**, 849–51.
32. Bowers, M.D. (1983). The role of iridoid glycosides in host-plant specificity of checkerspot butterflies. *Journal of Chemical Ecology*, **9**, 475–94.
33. Carter, M., Sachdev-Gupta, K., and Feeny, P. (1994). Tyramine, an oviposition stimulant for the black swallowtail butterfly from the leaves of wild parsnip. *Abstracts of the 11th Annual Meeting of the International Society of Chemical Ecology*, Syracuse, 55.

34. Chapman, R.F. (1982). Chemoreception: the significance of receptor numbers. *Advances in Insect Physiology*, **16**, 247–356.
35. Chapman, R.F. (2003). Contact chemoreception in feeding by phytophagous insects. *Annual Review of Entomology*, **48**, 455–84.
36. Chapman, R.F. and Bernays, E.A. (1989). Insect behavior at the leaf surface and learning as aspects of host plant selection. *Experientia*, **45**, 215–22.
37. Chapman, R.F. and Fraser, J. (1989). The chemosensory system of the monophagous grasshopper, *Bootettix argentatus* Bruner (Orthoptera: Acrididae). *International Journal of Insect Morphology and Embryology*, **18**, 111–18.
38. Chew, F.S. and Renwick, J.A.A. (1995). Chemical ecology of hostplant choice in *Pieris* butterflies. In *Chemical ecology of insects* (2nd edn) (ed. R.T. Cardé and W.J. Bell), pp. 214–38. Chapman & Hall, New York.
39. Clyne, P. J., Warr, C.G., and Carlson J.R. (2000). Candidate taste receptors in *Drosophila. Science*, **287**, 1830–4.
40. De Boer, G. (1993). Plasticity in food preference and diet-induced differential weighting of chemosensory information in larval *Manduca sexta. Journal of Insect Physiology*, **39**, 17–24.
41. De Candolle, A.P. (1804). *Essai sur les propriétés médicinales des plantes, comparées avec leurs formes extérieures et leur classification naturelle*. Didot Jeune, Paris.
42. De Vos, M., Van Oosten, V.R., Van Pelt, J.A., van Loon, L.C., Dicke, M., and Pieterse, C.M.J. (2004). Herbivore-induced resistance: differential effectiveness against a set of microbial pathogens in *Arabidopsis thaliana*. In *Biology of plant–microbe interactions*, Vol. 4 (ed. I. Tikhonovich, B. Lugtenberg, and N. Provorov), pp. 40–3. International Society for Molecular Plant–Microbe Interactions, St Paul, MN.
43. Del Campo, M.L., Miles, C.I., Schroeder, F.C., Müller, C., Booker, R., and Renwick, J.A.A. (2001). Host recognition by the tobacco hornworm is mediated by a host plant compound. *Nature*, **411**, 186–9.
44. Derridj, S., Gregoire, V., Boutin, J.P., and Fiala, V. (1989). Plant growth stages in the interspecific oviposition preference of the European corn borer and relations with chemicals present on the leaf surfaces. *Entomologia Experimentalis et Applicata*, **53**, 267–76.
45. Derridj, S., Fiala, V., and Boutin, J.P. (1991). Host plant oviposition preference of the European corn borer (*Ostrinia nubilalis* Hbn.). A biochemical explanation. *Symposia Biologica Hungarica*, **39**, 455–6.
46. Derridj, S., Wu, B.R., Stammitti, L., Garrec, J.P., and Derrien, A. (1996). Chemicals on the leaf surface, information about the plant available to insects. *Entomologia Experimentalis et Applicata*, **80**, 197–201.
47. Dethier, V.G. (1941). Chemical factors determining the choice of food by *Papilio* larvae. *American Naturalist*, **75**, 61–73.
48. Dethier, V.G. (1947). *Chemical insect attractants and repellents*. Blakiston, Philadelphia.
49. Dethier, V.G. (1973). Electrophysiological studies of gustation in lepidopterous larvae. II. Taste spectra in relation to food-plant discrimination. *Journal of Comparative Physiology*, **82**, 103–33.
50. Dethier, V.G. (1976). *The hungry fly*. Harvard University Press, Cambridge, MA.
51. Dethier, V.G. (1982). Mechanisms of host plant recognition. *Entomologia Experimentalis et Applicata*, **31**, 49–56.
52. Dethier, V.G. (1987). Analyzing proximate causes of behavior. In *Evolutionary genetics of invertebrate behavior* (ed. M.D. Huettel), pp. 319–28. Plenum Press, New York.
53. Dethier, V.G. and Crnjar, R.M. (1982). Candidate codes in the gustatory system of caterpillars. *Journal of General Physiology*, **79**, 549–69.
54. Dethier, V.G. and Kuch, J.H. (1971). Electrophysiological studies of gustation in lepidopterous larvae. I. Comparative sensitivity to sugars, amino acids, and glycosides. *Zeitschrift für Vergleichende Physiologie*, **72**, 343–63.
55. Doskotch, R.W., Mikhail, A.A., and Chatterjee, S.K. (1973). Structure of the water-soluble feeding stimulant for *Scolytus multistriatus*: a revision. *Phytochemistry*, **12**, 1153–5.
56. Dreyer, D.L. and Jones, K.C. (1981). Feeding deterrency of flavonoids and related phenolics towards *Schizaphis graminum* and *Myzus persicae*: aphid feeding deterrents in wheat. *Phytochemistry*, **20**, 2489–93.
57. Du, Y.-J., Van Loon, J.J.A., and Renwick, J.A.A. (1995). Contact chemoreception of oviposition stimulating glucosinolates and an oviposition deterrent cardenolide in two subspecies of *Pieris napi. Physiological Entomology*, **20**, 164–74.
58. Eigenbrode, S.D. (1996). Plant surface waxes and insect behaviour. In *Plant cuticles* (ed. G. Kerstiens), pp. 201–21. Bios Scientific Publishers, Oxford.
59. Eigenbrode, S.D. and Espelie, K.E. (1995). Effects of plant epicuticular lipids on insect herbivores. *Annual Review of Entomology*, **40**, 171–94.
60. Fabre, J.H. (1886). *Souvenirs entomologiques*, Vol. 3. Delagrave, Paris.

61. Feeny, P., Sachdev-Gupta, K., Rosenberry, L., and Carter, M. (1988). Luteolin 7-*O*-(6″-*O*-malonyl)-β-D-glucoside and *trans*-chlorogenic acid: oviposition stimulants for the black swallowtail butterfly. *Phytochemistry*, **27**, 3439–48.
62. Deleted.
63. Fraenkel, G.S. (1959). The raison d′être of secondary plant substances. *Science*, **129**, 1466–70.
64. Frazier, J.L. (1992). How animals perceive secondary plant compounds. In *Herbivores: their interactions with secondary plant metabolites*, Vol. 2 (2nd edn) (ed. G.A. Rosenthal and M.R. Berenbaum), pp. 89–133. Academic Press, New York.
65. Frazier, J.L. and Chyb, S. (1995). Use of feeding inhibitors in insect control. In *Regulatory mechanisms in insect feeding* (ed. R.F. Chapman and G. de Boer), pp. 364–81. Chapman & Hall, New York.
66. Gabrys, B., Tjallingii, W.F., and Van Beek, T.A. (1997). Analysis of EPG recorded probing by cabbage aphid on host plant parts with different glucosinolate contents. *Journal of Chemical Ecology*, **23**, 1661–73.
67. Garzo, E., Soria, C., Gomez-Guillamon, M.L., and Fereres, A. (2002). Feeding behavior of *Aphis gossypii* resistant accessions on different melon genotypes (*Cucumis melo*). *Phytoparasitica*, **30**, 129–40.
68. Givovich, A. and Niemeyer, H.M. (1991). Hydroxamic acids affecting barley yellow dwarf virus transmission by the aphid *Rhopalosiphum padi*. *Entomologia Experimentalis et Applicata*, **59**, 79–85.
69. Glendinning, J.I., Valcic, S., and Timmermann, B.N. (1998). Maxillary palps can mediate taste rejection of plant allelochemicals by caterpillars. *Journal of Comparative Physiology A*, **183**, 35–43.
70. Glendinning, J.I., Nelson, N.M., and Bernays, E.A. (2000). How do inositol and glucose modulate feeding in *Manduca sexta* caterpillars? *Journal of Experimental Biology*, **203**, 1299–315.
71. Glendinning J.I., Davis, A., and Ramaswamy, S. (2002). Contribution of different taste cells and signaling pathways to the discrimination of 'bitter' taste stimuli by an insect. *Journal of Neuroscience*, **22**, 7281–7.
72. Gregory, P., Avé, D.A., Bouthyette, P.J., and Tingey, W.M. (1986). Insect-defensive chemistry of potato glandular trichomes. In *Insects and the plant surface* (ed. B. Juniper and T.R.E. Southwood), pp. 173–83. Edward Arnold, London.
73. Griffiths, D.W., Deighton, N., Birch, A.N.E., Patrian, B., Baur, R., and Städler, E. (2001). Identification of glucosinolates on the leaf surface of plants from the Cruciferae and other closely related species. *Phytochemistry*, **57**, 693–700.
74. Guerra, A.A. and Shaver, T.N. (1969). Feeding stimulants from plants for larvae of the tobacco budworm and bolworm. *Journal of Economic Entomology*, **62**, 98–100.
75. Gupta, P.D. and Thorsteinson, A.J. (1960). Food plant relationships of the diamond-back moth (*Plutella maculipennis*). II. Sensory regulation of oviposition of the adult female. *Entomologia Experimentalis et Applicata*, **3**, 241–50.
76. Haley Sperling, J.L. and Mitchell, B.K. (1990). A comparative study of host recognition and the sense of taste in *Leptinotarsa*. *Journal of Experimental Biology*, **157**, 439–59.
77. Hamamura, Y., Hayashiya, K., Naito, K., Matsuura, K., and Nishida, J. (1962). Food selection by silkworm larvae. *Nature*, **194**, 754–5.
77a. Harborne, J.B. and Grayer, R.J. (1994). Flavonoids and insects. In *The flavonoids: advances in research since 1986* (ed. J.B. Harborne), pp. 589–618. Chapman & Hall, London.
78. Hare, J.D. and Elle, E. (2002). Variable impact of diverse insect herbivores on dimorphic *Datura wrightii*. *Ecology*, **83**, 2711–20.
79. Haribal, M. and Feeny, P. (1998). Oviposition stimulant for the zebra swallowtail butterfly, *Eurytides marcellus*, from the foliage of pawpaw, *Asimina triloba*. *Chemoecology*, **8**, 99–110.
80. Harris, M.O. and Miller, J.R. (1984). Foliar form influences ovipositional behaviour of the onion fly. *Physiological Entomology*, **9**, 145–55.
81. Hedin, P.A., Thompson, A.C., and Minyard, J.P. (1966). Constituents of the cotton bud. III. Factors that stimulate feeding by the cotton weevil. *Journal of Economic Entomology*, **59**, 181–5.
82. Hilker, M., Rohfritsch, O., and Meiners, T. (2002). The plant's response towards insect egg deposition. In *Chemoecology of insect eggs and egg deposition* (ed. M. Hilker and T. Meiners), pp. 205–33. Blackwell, Berlin.
83. Hoffmeister, T.S. and Roitberg, B.D. (2002). Evolutionary ecology of oviposition marking pheromones. In *Chemoecology of insect eggs and egg deposition* (ed. M. Hilker and T. Meiners), pp. 319–47. Blackwell, Berlin.
84. Honda, K. (1990). Identification of host-plant chemicals stimulating oviposition by swallowtail butterfly, *Papilio protenor*. *Journal of Chemical Ecology*, **16**, 325–37.
85. Honda, K. (1995). Chemical basis of differential oviposition by lepidopterous insects. *Archives of Insect Biochemistry and Physiology*, **30**, 1–23.
86. Honda, K., Tada, A., Hayashi, N., Abe, F., and Yamauchi, T. (1995). Alkaloidal oviposition stimulants for a danaid butterfly, *Ideopsis similis* L., from a

host plant, *Tylophora tanakae* (Asclepiadaceae). *Experientia*, **51**, 753–6.

87. Hsiao, T.H. (1985). Feeding behavior. In *Comprehensive insect physiology, biochemistry & pharmacology*, Vol. 9 (ed. G.A. Kerkut and L.I. Gilbert), pp. 497–512. Pergamon Press, New York.
88. Hsiao, T.H. (1988). Host specificity, seasonality and bionomics of *Leptinotarsa* beetles. In *Biology of Chrysomelidae* (ed. P. Jolivet, E. Petitpierre, and T.H. Hsiao), pp. 581–99. Kluwer Academic, Dordrecht.
89. Huang, X. and Renwick, J.A.A. (1993). Differential selection of host plants by two *Pieris* species: the role of oviposition stimulants and deterrents. *Entomologia Experimentalis et Applicata*, **68**, 59–69.
90. Hurter, J., Ramp, T., Patrian, B., Städler, E., Roessingh, P., Baur, R., *et al*. (1999). Oviposition stimulants for the cabbage root fly: isolation from cabbage leaves. *Phytochemistry*, **51**, 377–82.
91. Isidoro, N., Solinas, M., Baur, R., Roessingh, P., and Städler, E. (1994). Ultrastructure of a tarsal sensillum of *Delia radicum* L. (Diptera: Anthomyiidae) sensitive to important host-plant compounds. *International Journal of Insect Morphology and Embryology*, **23**, 115–25.
92. Jackson, D.M., Severson, R.F., Johnson, A.W., Chaplin, J.F., and Stephenson, M.G. (1984). Ovipositional response of tobacco budworm moths (Lepidoptera: Noctuidae) to cuticular chemical isolates from tobacco leaves. *Environmental Entomology*, **13**, 1023–30.
93. Jeffree, C.E. (1986). The cuticle, epicuticular waxes and trichomes of plants, with reference to their structure, functions and evolution. In *Insects and the plant surface* (ed. B. Juniper and T.R.E. Southwood), pp. 23–64. Edward Arnold, London.
94. Jermy, T. (1958). Untersuchungen über Auffinden und Wahl der Nahrung beim Kartoffelkäfer (*Leptinotarsa decemlineata* Say). *Entomologia Experimentalis et Applicata*, **1**, 179–208.
95. Jermy, T. (1966). Feeding inhibitors and food preference in chewing phytophagous insects. *Entomologia Experimentalis et Applicata*, **9**, 1–12.
96. Jermy, T. (1993). Evolution of insect–plant relationships—a devil's advocate approach. *Entomologia Experimentalis et Applicata*, **66**, 3–12.
97. Jermy, T. (1994). Hypotheses on oligophagy: how far the case of the Colorado potato beetle supports them. In *Novel aspects of the biology of Chrysomelidae* (ed. P.H. Jolivet, M.L. Cox, and E. Petitpierre), pp. 127–39. Kluwer Academic, Dordrecht.
98. Jermy, T. and Szentesi, Á. (1978). The role of inhibitory stimuli in the choice of oviposition site by phytophagous insects. *Entomologia Experimentalis et Applicata*, **24**, 458–71.
99. Jördens-Röttger, D. (1979). Das Verhalten der schwarzen Bohnenblattlaus *Aphis fabae* Scop. gegenüber chemische Reizen von Pflanzenoberflächen. *Zeitschrift für Angewandte Entomologie*, **88**, 158–66.
100. Juniper, B.E. and Jeffree, C.E. (1983). *Plant surfaces*. Edward Arnold, London.
101. Juvik, J.A., Babka, B.A., and Timmermann, E.A. (1988). Influence of trichome exudates from species of *Lycopersicon* on oviposition behavior of *Heliothis zea* (Boddie). *Journal of Chemical Ecology*, **14**, 1261–78.
102. Kennedy, J.S. (1987). Animal motivation: the beginning of the end? In *Advances in chemoreception and behaviour* (ed. R.F. Chapman, E.A. Bernays, and J.G. Stoffolano), pp. 17–31. Springer, New York.
103. Kennedy, J.S. and Booth, C.O. (1951). Host alternation in *Aphis fabae* Scop. I. Feeding preferences and fecundity in relation to the age and kind of leaves. *Annals of Applied Biology*, **38**, 25–65.
104. Kent, K.S. and Hildebrand, J.G. (1987). Cephalic sensory pathways in the central nervous system of *Manduca sexta* (Lepidoptera: Sphingidae). *Philosophical Transactions of the Royal Society, London*, **B315**, 3–33.
105. Klingauf, F. (1971). Die Wirkung des Glucosids Phlorizin auf das Wirtswahlverhalten von *Rhopalosiphum insertum* (Walk.) und *Aphis pomi* de Geer (Homoptera: Aphididae). *Zeitschrift für Angewandte Entomologie*, **68**, 41–55.
106. Klingauf, F., Nöcker-Wenzel, K., and Röttger, U. (1978). Die Rolle peripherer Pflanzenwachse für den Befall durch phytophage Insekten. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, **85**, 228–37.
107. Kubo, I., Lee, Y.-W., Balogh-Nair, V., Nakanishi, K., and Chapya, A. (1976). Structure of ajugarins. *Journal of the Chemical Society—Chemical Communications*, **1976**, 949–50.
108. Lam, W.-K.F. and Pedigo, L.P. (2001). Effect of trichome density on soybean pod feeding by adult bean leaf beetles (Coleoptera: Chrysomelidae). *Journal of Economic Entomology*, **94**, 1459–63.
109. Levy, E.C., Ishaaya, I., Gurevitz, E., Cooper, R., and Lavie, D. (1974). Isolation and identification of host compounds eliciting attraction and bite stimuli in the fruit bark beetle, *Scolytus mediterraneus* (Col., Scolytidae). *Journal of Agricultural and Food Chemistry*, **22**, 376–9.
110. Lin, S., Binder, B.F., and Hart, E.R. (1998). Insect feeding stimulants from the surface of Populus. *Journal of Chemical Ecology*, **24**, 1781–90.
111. Lombarkia, N. and Derridj, S. (2002). Incidence of apple fruit and leaf surface metabolites on *Cydia pomonella* oviposition. *Entomologia Experimentalis et Applicata*, **104**, 79–86.

112. Luo, L.-E., van Loon, J.J.A., and Schoonhoven, L.M. (1995). Behavioural and sensory responses to some neem compounds by *Pieris brassicae* larvae. *Physiological Entomology*, **20**, 134–40.
113. Lupton, F.G.H. (1967). The use of resistant varieties in crop protection. *World Review of Pest Control*, **6**, 47–58.
114. Ma, W.-C. (1972). Dynamics of feeding responses in *Pieris brassicae* Linn as a function of chemosensory input: a behavioural, ultrastructural and electrophysiological study. *Mededelingen Landbouwhogeschool Wageningen*, **72-11**, 1–162.
115. Ma, W.-C. and Schoonhoven, L.M. (1973). Tarsal chemosensory hairs of the large white butterfly *Pieris brassicae* and their possible role in oviposition behaviour. *Entomologia Experimentalis et Applicata*, **16**, 343–57.
116. Malakar, R. and Tingey, W.M. (2000). Glandular trichomes of *Solanum berthaultii* and its hybrids with potato deter oviposition and impair growth of potato tuber moth. *Entomologia Experimentalis et Applicata*, **94**, 249–57.
117. Marazzi, C. and Städler, E. (2004a). *Arabidopsis thaliana* leaf-surface extracts are detected by the cabbage root fly (*Delia radicum*) and stimulate oviposition. *Physiological Entomology*, **29**, 192–8.
118. Marazzi, C., Patrian, B., and Städler, E. (2004b). Secondary metabolites of the leaf surface affected by sulphur fertilisation and perceived by the diamondback moth. *Chemoecology*, **14**, 81–6.
119. Matsuda, K. and Matsuo, H. (1985). A flavonoid, luteolin-7-glucoside, as well as salicin and populin, stimulating the feeding of leaf beetles attacking salicaceous plants. *Applied Entomology and Zoology*, **20**, 305–13.
120. McIver, S.B. (1985). Mechanoreception. In *Comprehensive insect physiology, biochemistry & pharmacology*, Vol. 6 (ed. G.A. Kerkut & L.I. Gilbert), pp. 71–132. Pergamon Press, New York.
121. Matsumoto, Y. and Thorsteinson, A.J. (1968). Effect of organic sulfur comounds on oviposition in onion maggot *Hylemya antiqua* Meigen (Diptera, Anthomyiidae). *Applied Entomology and Zoology*, **3**, 5–12.
122. Matsumoto, Y. (1969). Some plant chemicals influencing the insect behaviors. *Proceedings of the 11th International Congress of Botany*, Seattle, p. 143.
123. Medeiros, L. and Moreira Gilson, R.P. (2002). Moving on hairy surfaces: modifications of *Gratiana spadicea* larval legs to attach on its host plant *Solanum sisymbriifolium*. *Entomologia Experimentalis et Applicata*, **102**, 295–305.
124. Menken, S.B.J. (1996). Pattern and process in the evolution of insect–plant associations: *Yponomeuta* as an example. *Entomologia Experimentalis et Applicata*, **80**, 297–305.
125. Menken, S.B.J., Herrebout, W.M., and Wiebes, J.T. (1992). Small ermine moths (*Yponomeuta*): their host relations and evolution. *Annual Review of Entomology*, **37**, 41–66.
126. Messchendorp, L., Van Loon, J.J.A., and Gols, G.J.Z. (1996). Behavioural and sensory responses to drimane antifeedants in *Pieris brassicae* larvae. *Entomologia Experimentalis et Applicata*, **79**, 195–202.
127. Messchendorp, L., Smid, H., and Van Loon, J.J.A. (1998). Role of an epipharyngeal sensillum in the perception of deterrents by Colorado potato beetle larvae. *Journal of Comparative Physiology A*, **183**, 255–64.
128. Metcalf, R.L., Metcalf, R.A., and Rhodes, A.M. (1980). Cucurbitacins as kairomones for diabroticite beetles. *Proceedings of the National Academy of Sciences of the USA*, **77**, 3769–72.
129. Miller, J.R. and Strickler, K.L. (1984). Finding and accepting host plants. In *Chemical ecology of insects* (ed. W.J. Bell and R.T. Cardé), pp. 127–57. Chapman & Hall, London.
130. Mitchell, B.K. (1974). Behavioural and electrophysiological investigations on the responses of larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*) to amino acids. *Entomologia Experimentalis et Applicata*, **17**, 255–64.
131. Mitchell, B.K. (1988). Adult leaf beetles as models for exploring the chemical basis of host-plant recognition. *Journal of Insect Phsyiology*, **34**, 213–25.
132. Mitchell, B.K. and Gregory, P. (1979). Physiology of the maxillary sugar sensitive cell in the red turnip beetle, *Entomoscelis americana*. *Journal of Comparative Physiology*, **132**, 167–78.
133. Mitchell, B.K. and Harrison, G.D. (1984). Characterization of galeal chemosensilla in the adult Colorado beetle, *Leptinotarsa decemlineata*. *Physiological Entomology*, **9**, 49–56.
134. Mitchell, B.K. and Itagaki, H. (1992). Interneurons of the subesophageal ganglion of *Sarcophaga bullata* responding to gustatory and mechanosensory stimuli. *Journal of Comparative Physiology A*, **171**, 213–30.
135. Mitchell, B.K. and Schoonhoven, L.M. (1974). Taste receptors in Colorado beetle larvae. *Journal of Insect Physiology*, **20**, 1787–93.
136. Mitchell, B.K., Rolseth, B.M., and McCashin, B.G. (1990). Differential responses of galeal gustatory sensilla of the adult Colorado potato beetle, *Leptinotarsa decemlineata* (Say), to leaf saps from host and non-host plants. *Physiological Entomology*, **15**, 61–72.

137. Montgomery, M.E. and Arn, H. (1974). Feeding response of *Aphis pomi*, *Myzus persicae* and *Amphorophora agathonica* to phlorizin. *Journal of Insect Physiology*, **20**, 413–21.
138. Morgan, E.D. and Mandava, N.B. (1990). *CRC Handbook of natural pesticides*, Vol. 6, *Insect attractants and repellents*. CRC Press, Boca Raton.
139. Morris, B.D., Foster, S.P., and Harris, M.O. (2000). Identification of 1-octacosanal and 6-methoxy-2-benzoxazolinone from wheat as ovipositional stimulants for Hessian fly, *Mayetiola destructor. Journal of Chemical Ecology*, **26**, 859–73.
140. Müller, C., and Renwick, J.A.A. (2001). Different phagostimulants in potato foliage for *Manduca sexta* and *Leptinotarsa decemlineata*. *Chemoecology*, **11**, 37–41.
141. Mullin, C.A., Chyb, S., Eichenseer, H., Hollister, B., and Frazier, J.L. (1994). Neuroreceptor mechanisms in insect gustation: a pharmacological approach. *Journal of Insect Physiology*, **40**, 913–31.
142. Nair, K.S.S. and McEwen, F.L. (1976). Host selection by the adult cabbage maggot, *Hylemya brassicae* (Diptera: Anthomyiidae): effect of glucosinolates and common nutrients on oviposition. *Canadian Entomologist*, **108**, 1021–30.
143. Nelson, N. and Bernays, E.A. (1998). Inositol in two host plants of *Manduca sexta*. *Entomologia Experimentalis et Applicata*, **88**, 189–93.
144. Nielsen, J.K., Larsen, L.M., and Sørensen, H. (1979). Host plant selection of the horseradish flea beetle, *Phyllotreta armoraciae* (Coleoptera: Chrysomelidae): identification of two flavonol glycosides stimulating feeding in combination with glucosinolates. *Entomologia Experimentalis et Applicata*, **26**, 40–8.
145. Deleted.
146. Nishida, R. (1995). Oviposition stimulants of swallowtail butterflies. In *Swallowtail butterflies: their ecology and evolutionary biology* (ed. J.M. Scriber, Y. Tsubaki, and R.C. Lederhouse), pp. 17–26. Scientific Publishers, Gainesville, FL.
147. Nishida, R. and Fukami, H. (1989). Oviposition stimulants of an Aristolochiceae-feeding swallowtail butterfly, *Atrophaneura alcinous*. *Journal of Chemical Ecology*, **15**, 2565–75.
148. Nishijima, Y. (1960). Host plant preference of the soybean pod borer, *Grapholita glicinivorella* (Matsumura) (Lep., Encosmidae). *Entomologia Experimentalis et Applicata*, **3**, 38–47.
149. Nottingham, S.F., Son, K.C., Wilson, D.D., Severson, R.F., and Kays, S.J. (1989). Feeding and oviposition preferences of sweet potato weevils, *Cyclas formicarius elegantulus* (Summers), on storage roots of sweet potato cultivars with differing surface chemistries. *Journal of Chemical Ecology*, **15**, 895–903.
150. Nufio, C.R. and Papaj, D.R. (2001). Host marking behavior in phytophagous insects and parasitoids. *Entomologia Experimentalis et Applicata*, **99**, 273–93.
151. Ohta, K.C., Matsuda, K., and Matsumoto, Y. (1998). Feeding stimulation of strawberry leaf beetle, *Galerucella vittaticollis* Baly (Coleoptera: Chrysomelidae) by quercetin glycosides in polygonaceous plants. *Japanese Journal of Applied Entomology and Zoology*, **42**, 45–9.
152. Panda, N. and Khush, G.S. (1995). *Host plant resistance to insects*. CAB International, London.
153. Panzuto, M. and Albert, P.J. (1998). Chemoreception of amino acids by female fourth- and sixth-instar larvae of the spruce budworm. *Entomologia Experimentalis et Applicata*, **86**, 89–96.
154. Pereyra, P.C. and Bowers, M.D. (1988). Iridoid glycosides as oviposition stimulants for the buckeye butterfly, *Junonia coenia* (Nympalidae). *Journal of Chemical Ecology*, **14**, 917–28.
155. Peterson, S.C., Herrebout, W.M., and Kooi, R.E. (1990). Chemosensory basis of host colonization by small ermine moth larvae. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen*, **93**, 287–94.
156. Peterson, S.C., Hanson, F.E., and Warthen, J.D. (1993). Deterrence coding by a larval *Manduca* chemosensory neurone mediating rejection of a non-host plant, *Canna generalis* L. *Physiological Entomology*, **18**, 285–95.
157. Deleted.
158. Quiras, C.F., Stevens, M.A., Rick, C.M., and Kok–Yokomi, M.K. (1977). Resistance in tomato to the pink form of the potato aphid, *Macrosiphum euphorbiae* (Thomas): the role of anatomy, epidermal hairs and foliage composition. *Journal of the American Society of Horticultural Science*, **102**, 166–71.
159. Raffa, K. (1987). Influence of host plant on deterrence by azadirachtin of feeding by fall armyworm larvae (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, **80**, 384–7.
160. Ranger, C.M., Backus, E.A., Winter, R.E.K., Rottinghaus, G.E., Ellersieck, M.R., and Johnson, D.W. (2004). Glandular trichome extracts from *Medicago sativa* deter settling by the potato leafhopper *Empoasca fabae*. *Journal of Chemical Ecology*, **30**, 927–43.
161. Rees, C.J.C. (1969). Chemoreceptor specificity associated with choice of feeding site by the beetle, *Chrysolina brunsvicensis* on its foodplant, *Hypericum*

hirsutum. *Entomologia Experimentalis et Applicata*, **12**, 565–83.

162. Reifenrath, K., Riederer, M., and Müller, C. (2005). Leaf surfaces of Brassicaceae lack feeding stimulants for *Phaedon cochleariae*. *Entomologia Experimentalis et Applicata*, **115**, 41–50.
163. Renwick, J.A.A. (1990). Oviposition stimulants and deterrents. In *Handbook of natural pesticides*, Vol. 6, *Insect attractants and repellents* (ed. E.D. Morgan and N.B. Mandava), pp. 151–60. CRC Press, Boca Raton, FL.
164. Renwick, J.A.A., Radke, C.D., Sachdev-Gupta, K., and Städler, E. (1992). Leaf surface chemicals stimulating oviposition by *Pieris rapae* (Lepidoptera: Pieridae). *Chemoecology*, **3**, 33–8.
165. Roessingh, P. and Städler, E. (1990). Foliar form, colour and surface characteristics influence oviposition behaviour in the cabbage root fly, *Delia radicum*. *Entomologia Experimentalis et Applicata*, **57**, 93–100.
166. Roessingh, P., Städler, E., Schöni, R., and Feeny, P. (1991). Tarsal contact chemoreceptors of the black swallowtail butterfly *Papilio polyxenes*: responses to phytochemicals from host- and non-host plants. *Physiological Entomology*, **16**, 485–95.
167. Roessingh, P., Städler, E., Fenwick, G.R., Lewis, J.A., Nielsen, J.K., Hurter, J., *et al*. (1992a). Oviposition and tarsal chemoreceptors of the cabbage root fly are stimulated by glucosinolates and host plant extracts. *Entomologia Experimentalis et Applicata*, **65**, 267–82.
168. Roessingh, P., Städler, E., Hurter, J., and Ramp, T. (1992b). Oviposition stimulant for the cabbage root fly: important new cabbage leaf surface compound and specific tarsal receptors. In *Proceedings of the 8th International Symposium on Insect–Plant Relationships* (ed. S.B.J. Menken, J.H. Visser, and P. Harrewijn), pp. 141–2. Kluwer Academic, Dordecht.
169. Roessingh, P., Städler, E., Baur, R., Hurter, J., and Ramp, T. (1997). Tarsal chemoreceptors and oviposition behaviour of the cabbage root fly (*Delia radicum*) sensitive to fractions and new compounds of host-leaf surface extracts. *Physiological Entomology*, **22**, 140–8.
170. Rogers, S.M. and Newland, P.L. (2003). The neurobiology of taste in insects. *Advances in Insect Physiology*, **31**, 141–204.
171. Roininen, H., Price, P.W., Julkunen-Tiitto, R., Tahvanainen, J., and Ikonen, A. (1999). Oviposition stimulant for a gall-inducing sawfly, *Euura lasiolepis*, on willow is a phenolic glucoside. *Journal of Chemical Ecology*, **25**, 943–53.
172. Roitberg, B.D. and Prokopy, R.J. (1987). Insects that mark host plants. *BioScience*, **37**, 400–6.
173. Romeis, J., Shanower, T.G., and Peter, A.J. (1999). Trichomes on pigeonpea (*Cajanus cajan* (L.) Millsp.) and two wild *Cajanus* spp. *Crop Science*, **39**, 564–9.
174. Rothschild, M. and Schoonhoven, L.M. (1977). Assessment of egg load by *Pieris brassicae* (Lepidoptera: Pieridae). *Nature*, **266**, 352–5.
175. Sachdev-Gupta, K., Feeny, P., and Carter, M. (1993). Oviposition stimulants for the pipevine swallowtail, *Battus philenor*, from an *Aristolochia* host plant: synergism between inositols, aristolochic acids and a monogalactosyl diglyceride. *Chemoecology*, **4**, 19–28.
176. Schoonhoven, L.M. (1967). Chemoreception of mustard oil glucosides in larvae of *Pieris brassicae*. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen*, **C70**, 556–68.
177. Schoonhoven, L.M. (1969). Amino-acid receptor in larvae of *Pieris brassicae*. *Nature*, **221**, 1268.
178. Schoonhoven, L.M. (1972). Secondary plant substances and insects. *Recent Advances in Phytochemistry*, **5**, 197–224.
179. Schoonhoven, L.M. (1973). Plant recognition by lepidopterous larvae. *Symposia of the Royal Entomological Society of London*, **6**, 87–99.
180. Schoonhoven, L.M. (1981). Chemical mediators between plants and phytophagous insects. In *Semiochemicals: their role in pest control* (ed. D.A. Nordlund, R.L. Jones, and W.J. Lewis), pp. 31–50. John Wiley, New York.
181. Schoonhoven, L.M. (1982). Biological aspects of antifeedants. *Entomologia Experimentalis et Applicata*, **31**, 57–69.
182. Schoonhoven, L.M. (1987). What makes a caterpillar eat? The sensory code underlying feeding behaviour. In *Advances in chemoreception and behaviour* (R.F. Chapman, E.A. Bernays, and J.G. Stoffolano), pp. 69–97. Springer, New York.
183. Schoonhoven, L.M. (1990). Host-marking pheromones in Lepidoptera, with special reference to *Pieris* spp. *Journal of Chemical Ecology*, **16**, 3043–52.
184. Schoonhoven, L.M. (1991). 100 years of botanical instinct. *Symposia Biologica Hungarica*, **39**, 3–14.
185. Schoonhoven, L.M. (1997). Verschaffelt 1910: a founding paper in the field of insect–plant relationships. *Proceedings Koninklijke Nederlandse Akademie van Wetenschappen*, **100**, 355–61.
186. Schoonhoven, L.M. and Blom, F. (1988). Chemoreception and feeding behaviour in a caterpillar: towards a model of brain functioning in insects. *Entomologia Experimentalis et Applicata*, **49**, 123–9.

187. Schoonhoven, L.M. and Derksen–Koppers, I. (1976). Effects of some allelochemics on food uptake and survival of a polyphagous aphid, *Myzus persicae. Entomologia Experimentalis et Applicata*, **19**, 52–6.

188. Schoonhoven, L.M. and Dethier, V.G. (1966). Sensory aspects of host-plant discrimination by lepidopterous larvae. *Archives Néerlandais de Zoologie*, **16**, 497–530.

189. Schoonhoven, L.M. and Luo, L.E. (1994). Multiple mode of action of the feeding deterrent, toosendanin, on the sense of taste in *Pieris brassicae* larvae. *Journal of Comparative Physiology A*, **175**, 519–24.

190. Schoonhoven, L.M., and van Loon, J.J.A. (2002). An inventory of taste in caterpillars: each species its own key. *Acta Zoologica Academiae Scientiarium Hungaricae*, **48(Suppl 1)**, 215–63.

191. Schoonhoven, L.M., Blaney, W.M., and Simmonds, M.S.J. (1992). Sensory coding of feeding deterrents in phytophagous insects. In *Insect–plant interactions*, Vol. 4 (ed. E.A. Bernays), pp. 59–79. CRC Press, Boca Raton.

192. Shields, V.D.C. and Mitchell, B.K. (1995a). Sinigrin as a feeding deterrent in two crucifer feeding, polyphagous lepidopterous species and the effects of feeding stimulant mixtures on deterrency. *Philosophical Transactions of the Royal Society, London*, **B347**, 439–46.

193. Shields, V.D.C. and Mitchell, B.K. (1995b). The effect of phagostimulant mixtures on deterrent receptor(s) in two crucifer-feeding lepidopterous species. *Philosophical Transactions of the Royal Society, London*, **B347**, 459–64.

194. Simmonds, M.S.J. and Blaney, W.M. (1991). Gustatory codes in lepidopterous larvae. *Symposia Biologica Hungarica*, **39**, 17–27.

195. Simmonds, M.S.J. and Blaney, W.M. (1996). Azadirachtin: advances in understanding its activity as an antifeedant. *Entomologia Experimentalis et Applicata*, **80**, 23–6.

196. Simmonds, M.S.J., Blaney, W.M., Mithen, R., Birch, A.N., and Fenwick, R. (1994). Behavioural and chemosensory responses of the turnip root fly (*Delia floralis*) to glucosinolates. *Entomologia Experimentalis et Applicata*, **71**, 41–57.

197. Simmonds, M.S.J., Blaney, W.M., Ley, S.V., Anderson, J.C., Banteli, R., Denholm, A.A., *et al.* (1995). Behavioural and neurophysiological responses of *Spodoptera littoralis* to azadirachtin and a range of synthetic analogues. *Entomologia Experimentalis et Applicata*, **77**, 69–80.

198. Singer, M.C., Thomas, C.D., and Parmesan, C. (1993). Rapid human-induced evolution of insect–host associations. *Nature*, **366**, 861–3.

199. Smith, B.D. (1966). Effect of the plant alkaloid sparteine on the distribution of the aphid *Acyrtosiphon spartii* (Koch). *Nature*, **212**, 213–14.

200. Smith, C.M. (1989). *Plant resistance to insects. A fundamental approach*. John Wiley, New York.

201. Städler, E. (1978). Chemoreception of host plant chemicals by ovipositing females of *Delia (Hylemya) brassicae. Entomologia Experimentalis et Applicata*, **24**, 711–20.

202. Städler, E. (1986). Oviposition and feeding stimuli in leaf surface waxes. In *Insects and the plant surface* (ed. B. Juniper and T.R.E. Southwood), pp. 105–21. Edward Arnold, London.

203. Städler, E. (1992). Behavioral responses of insects to plant secondary compounds. In *Herbivores: their interactions with secondary plant metabolites*, Vol. 2 (2nd edn) (ed. G.A. Rosenthal and M.R. Berenbaum), pp. 45–88. Academic Press, New York.

204. Städler, E. (2002). Plant chemical cues important for egg deposition by herbivorous insects. In *Chemoecology of insect eggs and egg deposition* (ed. M. Hilker and T. Meiners), pp. 171–204. Blackwell, Berlin.

205. Städler, E. and Buser, H.R. (1984). Defense chemicals in leaf surface wax synergistically stimulate oviposition by a phytophagous insect. *Experientia*, **40**, 1157–9.

206. Städler, E. and Buser, H.R. (1984). Defense chemicals in leaf surface wax synergistically stimulate oviposition by a phytophagous insect. *Experientia*, **40**, 1157–9.

207. Städler, E. and Roessingh, P. (1991). Perception of surface chemicals by feeding and ovipositing insects. *Symposia Biologica Hungarica*, **39**, 71–86.

208. Städler, E., Ernst, B., Hurter, J., and Boller, E. (1994). Tarsal contact chemoreceptor for host marking pheromone of the cherry fruit fly, *Rhagoletis cerasi*: responses to natural and synthetic compounds. *Physiological Entomology*, **19**, 139–51.

209. Städler, E., Renwick, J.A.A., Radke, C.D., and Sachdev-Gupta, K. (1995). Tarsal contact chemoreceptor response to glucosinolates and cardenolides mediating oviposition in *Pieris rapae. Physiological Entomology*, **20**, 175–87.

210. Stoner, K.A. (1990). Glossy leaf wax and plant resistance to insects in *Brassica oleracea* under natural infestation. *Environmental Entomology*, **19**, 730–9.

211. Stork, N.E. (1980). Role of waxblooms in preventing attachment to *Brassicas* by the mustard beetle, *Phaedon cochleariae. Entomologia Experimentalis et Applicata*, **28**, 100–7.

212. Sutcliffe, J.F. and Mitchell, B.K. (1982). Characterization of galeal sugar and glucosinolate-sensitive cells in *Entomoscelis americana* adults. *Journal of Comparative Physiology A*, **146**, 393–400.

213. Takemura, M., Nishida, R., Mori, N., and Kuwahara, Y. (2002). Acylated flavonol glycosides as probing stimulants of a bean aphid, *Megoura crassicauda*, from *Vicia angustifolia*. *Phytochemistry*, **61**, 135–40.
214. Tanton, M.T. (1962). The effect of leaf toughness on the feeding of larvae of the mustard beetle, *Phaedon cochleariae* Fab. *Entomologia Experimentalis et Applicata*, **5**, 74–8.
215. Thompson, J.N. (1994). *The coevolutionary process*. University of Chicago Press, Chicago.
216. Thorsteinson, A.J. (1958). The chemotactic influence of plant constituents on feeding by phytophagous insects. *Entomologia Experimentalis et Applicata*, **1**, 23–7.
217. Tjallingii, W.F. (1995). Regulation of phloem sap feeding by aphids. In *Regulatory mechanisms in insect feeding* (ed. R.F. Chapman and G. de Boer), pp. 190–209. Chapman & Hall, New York.
218. Tjallingii, W.F. (2000). Comparison of AC and DC systems for electronic monitoring of stylet penetration activities by homopterans. In *Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior* (ed. G.P. Walker and E.A. Backus), pp. 41–69. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, MD.
219. Tjallingii, W.F. and Hogen Esch, T. (1993). Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. *Physiological Entomology*, **18**, 317–28.
220. Tjallingii, W.F. and Mayoral, A.M. (1992). Criteria for host-plant acceptance by aphids. In *Proceedings of the 8th International Symposium on Insect–Plant Relationships* (ed. S.B.J. Menken, J.H. Visser, and P. Harrewijn), pp. 280–2. Kluwer Academics, Dordrecht.
221. Van Drongelen, W. (1979). Contact chemoreception of host plant specific chemicals in larvae of various *Yponomeuta* species (Lepidoptera). *Journal of Comparative Physiology*, **134**, 265–2.
222. Van Drongelen, W. and Van Loon, J.J.A. (1980). Inheritance of gustatory sensitivity in F1 progeny of crosses between *Yponomeuta cagnagellus* and *Y. malinellus* (Lepidoptera). *Entomologia Experimentalis et Applicata*, **28**, 199–203.
223. Van Helden, M. and Tjallingii, W.F. (1993). The resistance of lettuce (*Lactuca sativa* L.) to *Nasonovia ribisnigri*: the use of electrical penetration graphs to locate the resistance factor(s) in the plant. *Entomologia Experimentalis et Applicata*, **66**, 53–8.
224. Van Loon, J.J.A. (1990). Chemoreception of phenolic acids and flavonoids in larvae of two species of *Pieris*. *Journal of Comparative Physiology A*, **166**, 889–99.
225. Van Loon, J.J.A. (1996). Chemosensory basis of feeding and oviposition behaviour in herbivorous insects: a glance at the periphery. *Entomologia Experimentalis et Applicata*, **80**, 7–13.
226. Van Loon, J.J.A. and Schoonhoven, L.M. (1999). Specialist deterrent chemoreceptors enable *Pieris* caterpillars to discriminate between chemically different deterrents. *Entomologia Experimentalis et Applicata*, **91**, 29–35.
227. Van Loon, J.J.A. and Van Eeuwijk, F.A. (1989). Chemoreception of amino acids in larvae of two species of *Pieris*. *Physiological Entomology*, **14**, 459–69.
228. Van Loon, J.J.A., Blaakmeer, A., Griepink, F.C., Van Beek, T.A., Schoonhoven, L.M., and De Groot, Æ. (1992). Leaf surface compound from *Brassica oleracea* (Cruciferae) induces oviposition by *Pieris brassicae* (Lepidoptera: Pieridae). *Chemoecology*, **3**, 39–44.
229. Deleted.
230. Van Loon, J.J.A., Wang, C.-Z., Nielsen, J.K., Gols, R., and Qiu, Y.-T. (2002). Flavonoids from cabbage are feeding stimulants for diamondback moth larvae additional to glucosinolates: chemoreception and behaviour. *Entomologia Experimentalis et Applicata*, **104**, 27–34.
231. Verschaffelt, E. (1910). The causes determining the selection of food in some herbivorous insects. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen*, **13**, 536–42.
232. Vrieling, K. and Derridj, S. (2003). Pyrrolizidine alkaloids in and on the leaf surface of *Senecio jacobaea* L. *Phytochemistry*, **64**, 1223–8.
233. Deleted.
234. Wensler, R.J.D. (1962). Mode of host selection by an aphid. *Nature*, **195**, 830–1.
235. White, P.R., Chapman, R.F., and Ascoli-Christensen, A. (1990). Interactions between two neurons in contact chemosensilla of the grasshopper *Schistocera americana*. *Journal of Comparative Physiology, A*, **167**, 431–6.
236. Woodhead, S., Galeffi, C., and Marini Betollo, G.B. (1982). *p*-Hydroxybenzaldehyde is a major constituent of the epicuticular wax of seedling *Sorghum bicolor*. *Phytochemistry*, **21**, 455–6.
237. Yencho, C.G. and Tingey, W.M. (1994). Glandular trichomes of *Solanum berthaultii* alter host preference of the Colorado potato beetle, *Leptinotarsa decemlineata*. *Entomologia Experimentalis et Applicata*, **70**, 217–25.
238. Zielske, A.F., Simons, J.N., and Silverstein, R.M. (1972). A flavone feeding stimulant in alligatorweed. *Phytochemistry*, **11**, 393–6.

CHAPTER 8

Host-plant selection: variation is the rule

Variation is a basic characteristic of life. Obvious levels of variability are between species, families, orders, phyla, kingdoms, etc., but a surprisingly large variability also exists within species. This applies not least to host-plant choices in herbivores. The host-plant range of an insect species is not a fixed and unchangeable property. Meticulous studies have shown that some individuals or even whole populations of an insect species may reject plants, although they belong to the 'normal' host-plant range of the species. Both host-plant ranges and preference ranking of acceptable plants appear to be often variable within and among populations, and the assumption that herbivorous insects possess fixed host preferences is wrong. Differences in propensity to find or accept certain host plants may be determined genotypically or may result from previous experience. The latter category includes associative learning and other types of learning. From the multitude of observations described in the literature it appears that phenotypic variation in host-plant preference and use among individuals or populations of herbivorous insects is common. Genetic differentiation in host preference is probably more common than the few reports on this phenomenon suggest.[49,59] On the basis of the still limited information, it is assumed that variation in host-selection behaviour or insect performance, usually involves both genotypic and experiential factors. Thus, each insect is an individual, which deviates from the mean and which possesses its own set of food preferences and aversions. In this view, 'aberrant' behaviour does not exist. This chapter focuses on phenotypic aspects of host-plant selection, and genotypic variation in that behaviour is discussed in Chapter 11 (Section 11.3).

8.1 Geographical variation

Numerous cases are known in which insects in different parts of their distribution area show differences in host-plant preference. In North America the leaf-mining moth *Phyllonorycter blancardella* is restricted to members of the genus *Malus*, whereas in Europe, its native distribution area, it has a considerably broader host-plant range and thrives on plants belonging to at least seven other genera of the Rosaceae.[85] A reversed situation is met in the cicadellid *Graphocephala ennahi*. Whereas indigenous Nearctic populations feed exclusively on *Rhododendron*, this species is polyphagous in Europe, where it established at the beginning of the twentieth century. Here this insect can be found on various plant species belonging to as many as 13 different plant families. An explanation could be that, after its introduction into the new region, this species has started an expansion of its host-plant range under the new ecological conditions, whereas founding principles may have caused host-range restriction in the above-mentioned *P. blancardella* in its newly occupied region.[120] Why the two species show opposite responses remains obscure. Whatever their causes may be, these examples show that an insect's host range may change after its introduction into a new region.

The occurrence of considerable variation in preference rankings of their host plants by different populations of the Colorado potato beetle is exemplified by the following observations. In southern parts of North America this insect feeds only on *Solanum rostratum* and *S. augustifolium*, and can barely survive on either *S. elaeagnifolium* or cultivated potato (*S. tuberosum*). Populations in Arizona, however, are uniquely adapted to *S. elaeagnifolium*, whereas beetles collected on cultivated potatoes in northern parts of the USA die on *S. elaeagnifolium*.[63] Populations in North Carolina flourish on *S. carolinense*, but those from more northern locations exhibit uniformly low survival on this host. Heritable variation in the ability to survive on *S. carolinense* exists both between and within beetle populations.[61] The host-range expansion of the Colorado potato beetle to include potato, as happened in the recent past, must have involved major genetic changes.[82]

Two further examples found among butterflies and grasshoppers are presented to illustrate the notion that interpopulational variations occur in all major insect taxa. The tiger swallowtail, *Papilio glaucus*, a truly polyphagous butterfly, covers most of the North American continent and feeds throughout its range on at least seven plant families, but in any one geographical area this species is restricted to a subset of these host plants. A *P. glaucus* strain from Florida feeds practically monophagously on *Magnolia virginiana*, whereas conspecifics in Ohio are polyphagous.[24] Based on such differences in food preferences and ability to utilize different host-plant species, several subspecies have been recognized.[25,118] *Schistocerca emarginata* is a grasshopper that must be classified as a polyphagous species, although some of its populations are clearly monophagous. The differences in feeding habits between populations are not simply the result of differences in the food available to the different populations, but are probably based on genetic differentiation between populations. Such host-associated populations may, depending on the degree of genetic isolation, represent host races. Genetic differences of this type may form the beginning of sympatric speciation via host race formation (see Section 11.2).[131]

Many more examples of geographical variation in host-plant use in various insect lineages, including Lepidoptera, Homoptera, Coleoptera, and Diptera, can be cited, all showing that, even though a species may be polyphagous over its geographical range as a whole, larvae from local populations may be true specialists.[54,136]

Regional differences in host-plant preference often reflect adaptations to local conditions. Local factors, such as the presence of a competitor for food, may exert a selection pressure, resulting in host-plant specialization. This is well exemplified by the host preferences of two closely related weevil species, *Larinus sturnus* and *L. jaceae*, that feed in the heads of some thistles and related knapweeds. When populations of the two insect species occur together, both species are represented by 'biotypes' that select different Cardueae species as breeding hosts (an insect biotype is an individual or population identical to conspecifcs in morphology, but showing genetically fixed physiological and/or behavioural differences). Although the two species have almost identical food niches, they avoid larval competition for food and space, possibly presenting a case of ecological character displacement (Table 8.1).[144] The causes of these

Table 8.1 Regional host-plant differences in two related weevil species, *Larinus sturnus* and *Larinus jaceae* (data from Zwölfer, 1970)[144]

Geographical area	*Larinus sturnus*	*Larinus jaceae*
Switzerland, Jura	*Centaurea scabiosa*	*Carduus nutans*
Switzerland, Wallis	*Centaurea scabiosa*	n.p.
Germany, Pfalz	n.p.	*Centaurea scabiosa*
France, Alsace	*Carduus nutans*	n.p.

Note: n.p., Insect species not present.

differences in host-plant preference are largely unknown, but it seems likely that genetic differences occur between geographically separated populations. In laboratory choice tests the differences in host preference reflecting their region of origin were retained in adult individuals of *L. sturnus*, collected in different areas, suggesting a genetic basis for differences in host selection. The insects (and, don't forget, the thistle species as well) possibly constitute a mosaic of locally different (sibling) species that have evolved into the present pattern of insect–plant association.[144,145] In other instances of geographically different insect biotypes, the possibility of regional quality differences between plants also cannot be excluded *a priori* as an important factor causing different host-plant choices.

As a proximate cause of differences in food-plant choices between populations it is conceivable that the sense organs in different populations are differently tuned and that behavioural differences can be traced back, at least to some extent, to different sensory inputs. Indeed, *Pieris napi* butterflies originating from different geographical regions (Europe and North America) show different sensory responses to various glucosinolates, compounds known to play a key role in host recognition (Fig. 8.1). These differences are probably related to differences in host-plant species between the two continents.[142]

Besides the intraspecific variation in host-plant preference found in most insect species studied, the *limits of variation* (i.e. the conservative nature of insect–host relationships) is often an important feature. The Colorado potato beetle presents such a case. Two North American and three European populations of this beetle were studied for similarities and dissimilarities between their behavioural responses to solanaceous host- and non-host-plant species. Despite the fact that populations in North America and in Europe have evolved separately for about 70 years (approximately 150 generations), significant differences in food-related behaviour appeared mostly among populations of the same continent. This indicates that the ecological differences (alternative host plants, natural enemies) between the two continents, that conceivably act as different selective pressures, have not caused basic behavioural changes so far.[134] This lack of major intercontinental differences is the more surprising because in the middle of last century a new chromosomal race appeared in the USA, spread rapidly across the continent, and hybridized with the original race. The new race is absent in Europe.[63]

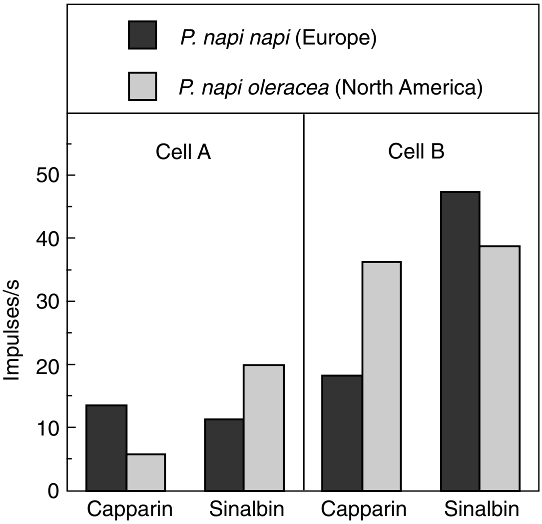

Figure 8.1 Sensitivity of chemoreceptor hairs on the tarsi of two *Pieris napi* subspecies for two glucosinolates. *P. n. napi* butterflies were collected in Europe, *P. n. oleracea* in North America. Nerve impulse frequencies are shown for two glucosinolate-sensitive cells (A and B) when stimulated with a solution of 100 μg/ml capparin or sinalbin. The two butterfly strains show significantly ($P < 0.05$) different responses to both stimuli for both taste cells. (Data from Du *et al.*, 1995.)[51]

8.2 Differences between populations in the same region

Sometimes quite striking differences in host preference exist even between different insect populations occurring short distances from one another. Adults of the nymphaline butterfly *Euphydryas editha* may oviposit on five different host species belonging to the Scrophulariaceae family in California. Some populations of this butterfly are strictly monophagous, whereas others are usually monophagous, but occasionally include a second host in their diet. A few populations exist in which the females oviposit on as many as four plant species, each belonging to a different genus. These interpopulation differences in breadth of host use are not caused primarily by differences in the availability of host species, because they occur in habitats with apparently more or less identical arrays of potential host species. Interestingly, within the oligophagous populations, some individuals are monophagous whereas others accept all potential host-plant species. The degree of host specialization in these populations appears to be a continuous variable.[125] Although under laboratory conditions host-plant preferences of this butterfly are less marked than in the field, the differences between populations as observed in the field are essentially maintained, again indicating a genetic basis for an extensive ecotypic variation in host use.[126] As mentioned above, it is conceivable that different insect populations display different host-plant preferences, (partly) caused by interpopulational variation among the plants.[55] Indeed, concomitant to genetic variation in *E. editha* butterflies, genetic variation has been found among their host-plant populations.[127,128]

Thus, conspecific plants occurring at different sites may be attacked by different insect species as a result of genetically determined differences in acceptability to these herbivores. Species with such regional differences, whether insects or plants, are obvious candidates for allopatric speciation.

8.3 Differences between individuals

Every person investigating the feeding responses of insects to different plant species now and then encounters individuals that show 'aberrant' behaviour and do not seem to follow the preferences dictated by the 'normal' host range of the species. These idiosyncrasies occur too often to consider them as mere abnormalities. When ignoring the extent of variation in behavioural or physiological parameters, as biologists often tend to do under the influence of Platonic philosophical traditions, essential information is lost. Such 'tyranny of the Golden Mean' disregards some basic principles of life.[11]

An illustration of 'deviant' behaviour was seen in an experiment with larvae of the privet hawkmoth *Sphinx ligustri*, which normally feeds on privet and some related Oleaceae. Ten caterpillars were removed from their common food and, after 1 h of starvation, each was placed on an oleander (*Nerium oleander*) leaf, with all leaves taken from the same bush. As expected, the caterpillars did not feed at all during a 4 h period, or at best took only a few test bites, with the exception of one individual. This insect soon started to eat and consumed 3.8 cm^2 of leaf surface during the rest of the experiment. Apparently, this individual did not possess a behavioural barrier to this particular plant, which belongs to the Apocynaceae, a plant family characterized by a copious latex flow when damaged. The highly poisonous cardenolides in this plant did not seem to affect the caterpillar, as the insect's growth and development were not markedly affected by the unusual meal. Observations of this type are certainly not rare events (Fig. 8.2).[112]

Likewise, the abundance of records of oviposition 'mistakes' by insects ovipositing on plants

Figure 8.2 Feeding activity of five tobacco hornworm larvae (*Manduca sexta*) that were each confined to one leaf of a non-host plant (dandelion) for a period of 4 h. All leaves were collected from the same plant. Three insects showed some nibbling (arrows) but refused to eat, whereas two insects started to feed immediately and consumed considerable amounts of leaf tissue. White leaf areas indicate parts consumed.

outside their normal host range[90,119] merit more attention than they usually receive. The occurrence of individuals with host choices that deviate from normal reflects random variation resulting from mutations. This variation is retained under natural selection to enable a species to cope with changing conditions in its environment.

The observation that generalist species make more oviposition errors than specialized species[117] may be attributed to the fact that ovipositing generalist females must deal with larger amounts of information. Their limited neural capacity for information processing may cause inaccuracies in their decisions.[14]

8.4 Environmental factors causing changes in host-plant preference

8.4.1 Seasonality

Host-plant preferences can vary with time among and within individuals in a population. Seasonal variation is pronounced in a number of aphid species that show an obligatory shifting between unrelated host-plant taxa over successive generations (Fig. 8.3).

This habit is typical of about 10% of all extant aphid species, although many non-alternating species are thought to have been derived from alternating ancestors.[77,87,121] Briefly, the life cycle of host-alternating species is as follows. Winged females leave the primary host plant in spring and their offspring spend the summer months on the secondary host plants, fast-growing herbaceous species. The tremendous risks of not finding an appropriate food plant after leaving the primary host seem to be compensated by improved food quality once a secondary host plant is found. Aphid species feeding on herbs achieve higher growth and reproduction rates per unit of sap energy consumed than tree-dwelling species (an average difference of as much as 160%).[81]

The better performance probably results from higher amounts of amino-nitrogen available in the phloem sap of herbs.[48] The summer generations consist of wingless, rapidly maturing, parthenogenetic individuals, which produce living young in quick succession. In early autumn winged forms, known as *sexuparae*, fly back to the primary host, on which the eggs are laid.

The summer and winter hosts often belong to quite distinct plant families (Table 8.2). The mealy plum aphid *Hyalopterus pruni*, for instance, feeds during the warm season on common reed (*Phragmites communis*) and withdraws during the winter

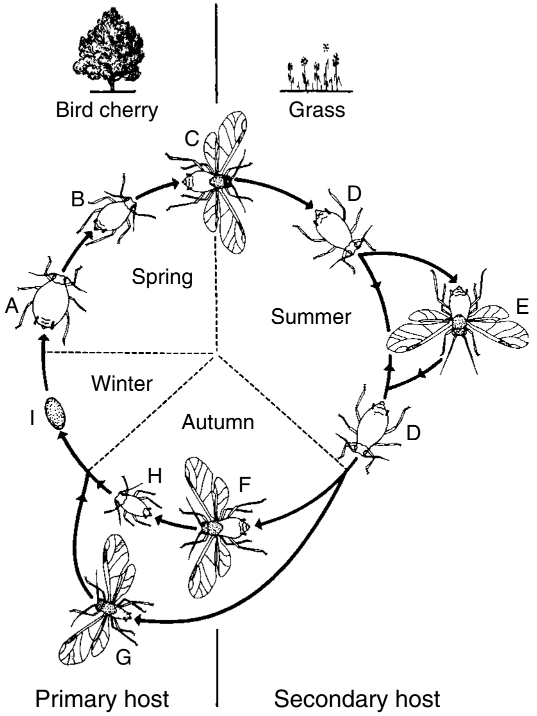

Figure 8.3 Life cycle of a heteroecious aphid species (bird cherry-oat aphid, *Rhopalosiphum padi*). A, fundatrix; B, apterous fundatrigenia; C, emigrant; D, apterous exule; E, alate exule; F, gynopara; G, male; H, ovipara; I, egg. (Reproduced from Dixon, 1973, with permission.)[47]

Table 8.2 Plant families of the primary and secondary host plants of three genera of host-alternating aphids (from Szelegiewicz, 1978, cited by Dixon, 1985)[48]

Aphid genus	Primary host plants	Secondary host plants
Rhopalosiphum	Rosaceae	Poaceae
Pemphigus	Salicaceae	Asteraceae, Poaceae, Apiaceae
Prociphilus	Caprifoliaceae, Oleaceae, Rosaceae	Pinaceae

to some *Prunus* species. What makes the aphids migrate in late summer? It appears that seasonal cues, such as photo-period, temperature, and cues related to the physiological condition of the plant,[84,87] govern the production of alternative morphs. These cues are apparently also involved in the behavioural switch with respect to host-plant selection. The ultimate factor causing the persistence of host alternation in a species is the possibility of exploiting the complementary growth patterns of woody and herbaceous plants.[77]

Seasonal changes in host preference occur in some other insect taxa. The cicad *Muellerianella fairmairei* is bivoltine in western Europe. It oviposits in spring on *Holcus lanatus* (Poaceae), but females of the second generation accept only *Juncus effusus* (Juncaceae) for egg-laying.[50] Other examples are found in some bivoltine lepidopterans, which may alternate between two entirely different host plants during successive generations. Thus, in the geometrid *Tephroclystis virgaureata*, caterpillars of the spring generation feed on some Asteraceae (e.g. *Solidago* and *Senecio*), whereas larvae of the summer generation occur on rosaceous plants (e.g. *Crataegus* and *Prunus*).[75] It would be interesting to know which factors govern the selection of oviposition sites by females of the two generations, but few attempts to analyse the causes of such changes in behaviour have been made. Seasonal factors may have changed the chemistry and/or nutritional value of potential host plants to such an extent that the insect switches from one plant species to another. Also, the insect's innate preferences may have changed.

In the case of another lepidopteran, the pipevine swallowtail *Battus philenor*, the seasonality of host-plant preference may be caused by changes on the part of the plant. The shift in host preferences occurs primarily through a change in the proportions of ovipositing females using two different search modes based on leaf shape. At any given time, some females alight preferably on narrow-leaved plants and neglect broad-leaved host plants, while others do the opposite. The butterflies appear to use a 'search image', as is also known in birds that learn to concentrate their foraging efforts on specific prey species.[138] Behavioural studies under experimental conditions have confirmed that most of the variation in search behaviour of this butterfly as seen in nature is probably due to differences in adult experience.[100]

8.4.2 Temperature

Food-plant preference sometimes changes with temperature. Colorado potato beetles offered a choice between potato and woody nightshade (*Solanum dulcamara*) normally show a predilection for potato. When choice experiments were performed at a temperature of 25°C or higher, however, the preference appeared to be reversed (Fig. 8.4).

Does the chemical composition of one or both plants change with temperature, affecting their sensory impressions on the insect? Or is the insect's behaviour modified by changes in its central nervous system or chemoreceptors? We do not know.

Another example of the influence of temperature on feeding habits concerns the alfalfa aphid *Terioaphis maculata*. Some alfalfa cultivars that are normally resistant to this insect become susceptible at a temperature of 10°C. Conceivably, reduced metabolic activity and lower mobility of the aphids at low temperatures are contributing factors,[109] but also in this case it is not known whether temperature modifies the insect, the plant, or both. Similar

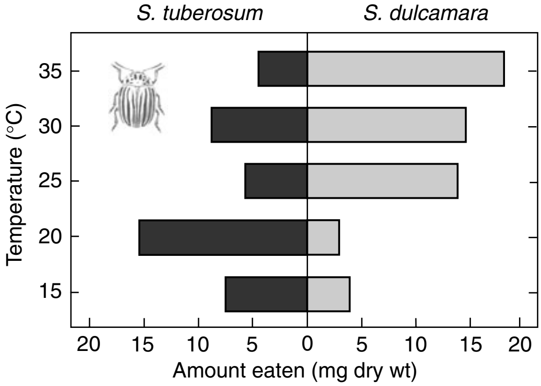

Figure 8.4 Food-plant preferences in Colorado potato beetles at different temperatures. Food consumption (mg eaten per beetle over 4 h) increases with temperature. Between 20 and 25°C, food preference switches from *Solanum tuberosum* to *Solanum dulcamara*. (Data from Bongers, 1970.)[23]

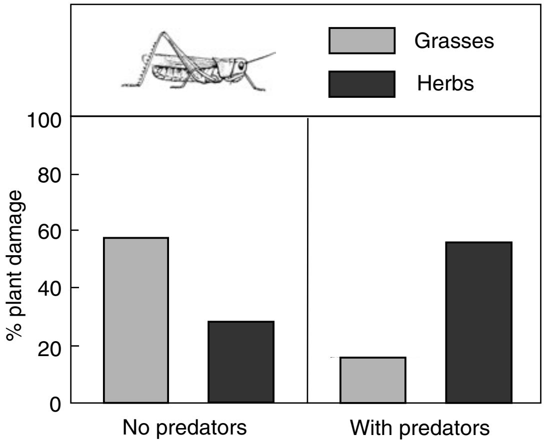

Figure 8.5 Damage inflicted on plants by grasshoppers (*Melanoplus femur-rubrum*) in the absence or presence of predatory spiders. When running a predation risk the insects decrease their dietary proportion of grass and eat more herbs. (Data from Beckerman *et al*., 1997.)[10]

observations were recorded for some other aphids, heteropterans, and Hessian flies (*Mayetiola destructor*), but the mechanisms involved remain obscure.[89]

8.4.3 Predation risks

Another extrinsic factor affecting food selection behaviour in herbivores may be found in the presence or absence of predators. In the absence of predatory spiders the red-legged grasshopper *Melanoplus femur-rubrum* feeds predominantly on grasses. However, when spiders are around, they clearly prefer to feed on herbs (Fig. 8.5). The structurally more complex herb vegetation may provide refuge from predators. Therefore this change in behaviour could reflect a shift in habitat selection rather than a shift in food preferences.[10] Some other examples are known of enemy avoidance behaviour as a result of learning.[45]

8.5 Internal factors causing changes in host-plant preference

Food plant preference of an individual insect does not always remain constant throughout its lifetime, but may change, for instance when nutritive needs change during ontogeny.

8.5.1 Developmental stage

In many herbivorous insect species the food-plant range becomes either narrower or wider during larval development. During their final instar many caterpillars accept more plant species than younger instars. Such diet broadening is quite striking in, for instance, larvae of the garden tiger moth *Arctia caja*. The list of food plants of their younger instars is rather short, whereas later instar caterpillars reject hardly any plant species.[86] There are also many cases of the reverse reported in the literature—of developing larvae that are much more fastidious eaters than newly hatched larvae. This could be the result of 'preference induction', a kind of learning behaviour discussed in Section 8.6.2.

Changes in food preference behaviour during development may be an expression of altered nutritional requirements. Indeed, there is no reason to expect *a priori* that the insect's nutritive needs will remain constant during ontogeny. To investigate possible changes in feeding preferences over time, gypsy moth larvae (*Lymantria dispar*) were reared with continuous access to two types of artificial diet. One diet contained a lower than optimal level of lipids; the other was low in protein content. From early to late instars, preference clearly shifted away from a lipid-deficient, high-protein diet towards protein-deficient, high-lipid food. This change in food selection probably reflects an adjustment to shifting nutritional demands.[129] The observation that larvae of this species in the wild often switch from plant to plant and may feed on several different plant species during development may, in view of the aforementioned experiment, be considered as nutrient intake optimization or self-selection (see below) related to changes in nutritional requirements.[5] In migratory locusts (*Locusta migratoria*), too, the relative requirements for protein and carbohydrate change during somatic growth of the adults. In this insect the neural responsiveness of chemosensilla on the maxillary palps appeared to be consistent with alterations in protein and sugar ingestion, indicating the presence of feedback from nutritional demands to receptor sensitivity.[124]

Some caterpillar species show a distinct switch to a different part of their host plant as they grow.

The reasons for such switches in feeding site are obscure. Nutritional factors or changes in predation risks with increasing size may exert selection pressure leading to a shift of place.[102] Seasonal changes in plant quality may also play a role, but could equally well reflect changes in the ability to accept alternative feeding sites due to morphological changes resulting from increased body size, for instance allowing feeding on tougher tissues.

Young instars of the native American butterfly *Pieris napi oleracea* refuse to feed on the introduced crucifer garlic mustard *Alliaria petiolata* due to the presence of a cyanopropenyl glycoside, alliarinoside, which causes post-ingestive toxicity. Later larval stages are insensitive to this compound but are deterred by another glycoside in the same plant, isovitexin-6″-D-β-glucopyranoside.[106] This example shows that sensitivity to identified plant-defensive compounds may change during larval development.

A quite spectacular change in feeding habits during larval development is exhibited by a number of lycaenid caterpillars. At first these species are vegetarian, but at some developmental stage the larvae stop eating and drop to the ground. They may then be adopted by ants and taken to the nest where they are placed among the ant brood. The 'myrmecophilous' ('ant-liking') larvae now produce, from special glands, secretions that contain up to 20% sugars and sometimes also small amounts of amino acids. These substances appease ants and, in many species, reward them for protecting the caterpillars against predators and parasitoids. The caterpillars, once inside the brood chamber of the ant nest, devour the fluid tissues of their hosts' offspring.[9,36] The bizarre change from herbivory to carnivory on ant brood as a normal pattern in many Lycaenidae bears some analogy to the cannibalistic habit that, under certain conditions, is exhibited even by several highly specialized herbivorous insects,[53] such as flour beetles (*Tribolium castaneum*),[143] the southwestern cornborer (*Diatraea grandiosella*),[135] and the milkweed leaf beetle (*Labodomera clivicollis*).[46]

Large differences in food preference may be found between the larval and adult stages of an insect. Larvae of the western corn rootworm *Diabrotica virgifera virgifera* are strictly monophagous, feeding only on corn roots, whereas the adult beetles are polyphagous.[31] As with many other biological traits, feeding behaviour may change drastically with the transition from larva to adult, owing to altered nutritional requirements and environmental conditions.

8.5.2 Insect sex affects food choice

In the above-cited experiments on dietary selection by gypsy moth larvae, a significant difference was noted (in addition to that between early-and late-instar larvae) between the dietary preferences of male and female larvae. Male larvae ate a higher proportion from the protein-deficient, high-lipid diet than female larvae. Clearly this reflects a physiological adjustment to the fact that in this species only male moths possess wings and develop the capability to fly. Lipids need to be stored to serve as fuel for flight. Females, on the other hand, require extra amounts of protein for egg development.[129] Hormonal differences between male and female larvae presumably control an accurate dietary balance of protein–lipid intake. In another, somewhat more natural, type of feeding trial, tropical walking sticks (*Lamponius portoricensis*) were offered leaves of four of their most common food-plant species. Male and female insects consumed different relative amounts of the various kinds of plant, thereby demonstrating foraging differences between the sexes, just as in the gypsy moth larvae.[107]

Observations in the field have also revealed dietary divergence between the sexes in a number of grasshopper species. Whereas in *Oedaleus senegalensis* males of any stage and immature females feed predominantly on the leaves of millet plants, mature females show a liking for the milky seed-heads of this plant, thereby satisfying their increased protein demands during oogenesis.[26] Another field study on 14 grasshopper species reported that diet composition and food preferences were significantly different between the two sexes. These findings lead to an interesting conclusion: male and female genotypes of the insects mentioned occupy different food niches.[141]

8.6 Experience-induced changes in host-plant preference

In contrast to vertebrates, the behaviour of herbivorous insects—both in general and particularly in terms of food-related behaviour—is primarily determined genetically. Nevertheless, different types of learning may significantly change feeding or oviposition behaviour. Two learning types can be distinguished: associative and non-associative learning. *Associative learning* is the ability to associate certain temporal relations between events. When an animal is exposed to a previously meaningless (ineffective) stimulus (the conditioned stimulus; CS) at the same time as a meaningful stimulus (the unconditioned stimulus; US) that produces either a positive or negative response and coincides with the CS within a certain time interval, it may from then on associate both stimuli. *Non-associative learning* involves simpler types of learning in which no coupling of CS and US exists. Habituation and induced food preferences are forms of non-associative learning, whereas food-aversion learning, dietary self-selection, and experience-induced changes in oviposition behaviour represent associative learning.[13,133]

8.6.1 Non-associative changes

(a) Habituation to deterrents

Habituation (or desensitization) is a waning of the responsiveness to a repeatedly presented stimulus.[80] It is considered to be the simplest form of learning.

As the specificity of plant acceptance by herbivorous insects is determined predominantly by the occurrence of secondary plant substances that inhibit feeding (see Section 7.6.1), habituation to deterrent stimuli has been studied in some detail. For example, freshly moulted fifth-instar nymphs of two acridid species, *Schistocerca gregaria* and *Locusta migratoria*, were divided into two groups. One group was reared on untreated sorghum leaves ('naive group'), the other ('experienced group') received sorghum leaves treated with the mildly deterrent nicotine hydrogen tartrate for 19 h and untreated food for 5 h per day. Each day, 10 naive and 10 experienced nymphs were given deterrent-treated sorghum leaves in a no-choice situation and the amount consumed during a period of 19 h was measured. Figure 8.6A shows that experienced nymphs of the polyphagous *S. gregaria* habituated to the deterrent: they consumed much more from the deterrent-treated leaf than naive nymphs. The nymphs of *L. migratoria*, a food specialist (Fig. 8.6B) also showed some habituation, albeit to a lesser degree.[68]

Habituation may occur to some chemicals, whereas others never become acceptable. Tobacco hornworm larvae, for example, rapidly habituate to the presence of salicin in their diet, but never adapt to food adulterated by small amounts of aristolochic acid.[58] Locusts were also found to habituate to relatively weak stimuli, but not to chemicals that inhibited feeding for more than 12 h.[69,132]

Insects do not habituate to non-host plants, even if they are just below the acceptance level.[67] The acceptance level of marginal host plants, however, may be increased after cross-habituation to certain feeding deterrents.[64] In the non-host plants of specialized insects it is probably the presence of a whole complex of deterrent stimuli that prevents habituation to the deterrent effect of these plants. This explanation is supported by the observation that several lepidopterous larvae, after repeated exposure, readily habituate to a single deterrent compound (azadirachtin) but not to plant extracts containing these as major components.[2,22]

Is habituation a process that takes place in the central nervous system, or are changes in the peripheral chemoreceptor system responsible for the increased acceptability of a deterrent? The observation that incorporation of the deterrent compound salicin into the diet of *Manduca sexta* larvae reduced sensitivity of the deterrent receptor cell to this compound suggests participation of the chemoreceptors in the learning process. A concomitant effect was that such larvae readily accepted salicin-treated host plants that were normally rejected.[111] These findings agree with the idea that, in addition to central learning processes, 'peripheral learning' takes place.[57,113] However, a study with nymphs of *Schistocerca gregaria* on the neural basis of habituation to the deterrent nicotine hydrogen tartrate (NHT) did not reveal any peripheral changes, suggesting that in this insect only

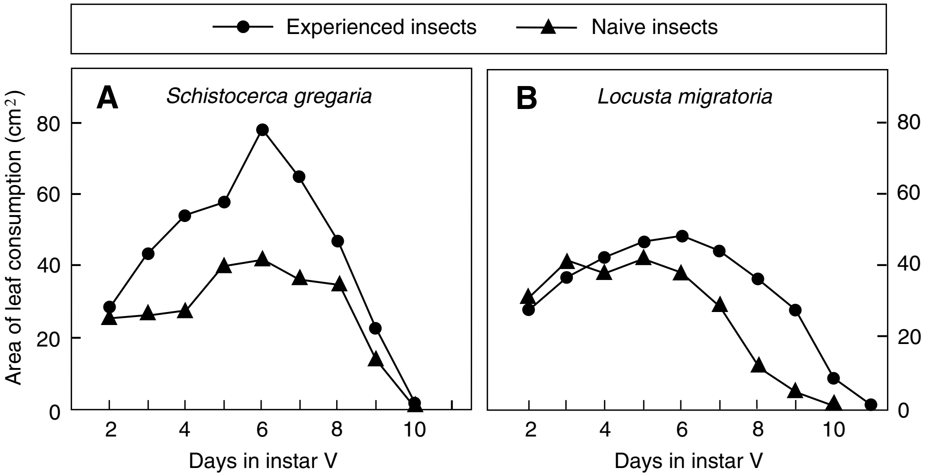

Figure 8.6 Quantities of deterrent-treated sorghum leaves consumed daily over the instar by experienced and naive fifth-instar nymphs of (**A**) the polyphagous desert locust *Schistocerca gregaria* and (**B**) the oligophagous migratory locust *Locusta migratoria*. (Redrawn from Jermy *et al.*, 1982.)[69]

central learning is involved. In an elegant experiment, small pieces of nylon tubing were fixed around the maxillary palpi. These appendages comprise 30% of all mouthpart chemoreceptors. An experimental group of insects received NHT solution in the tubes for several hours daily, while in the control group the tubes were filled with distilled water. After 4 days the nymphs were tested as follows: the tubes were left in place, but were empty, and leaves treated with NHT were given as food to both groups. The experimental group consumed significantly more of the NHT-treated leaves than the control group, despite the fact that direct perception of the chemical by the maxillary receptors was prevented by the tubes. These results clearly prove that habituation is mediated by the central nervous system; the information on NHT provided by the palpal receptors during the pretreatment was stored centrally and then compared with the information provided by the receptors on other mouthparts when the insects ate the NHT-treated leaves during the test.[132]

The question remains why insects with the capability to habituate to a deterrent compound do not do so in all instances. The answer may lie in the fact that some compounds, although deterrent, are physiologically harmless, whereas other deterrents are toxic. In the latter case the insect must, in addition to desensitizing the (peripheral or central) gustatory systems that mediate the aversive response, activate post-ingestive mechanisms that reduce its physiological sensitivity to the noxious compound. In cases where the detoxification system is unable to cope with the noxious chemicals, the insects will not habituate under penalty of death.[57]

(b) Induction of feeding preference

In an early book on entomology, Kirby and Spence[74] noted that, although insects may feed on various food plants, individuals of such generalist species, once they feed on a particular plant, often clearly show food specialization. 'It is worthy of remark, however, that when some of these have fed for a time on one plant they will die rather than eat another, which would have been perfectly acceptable to them if accustomed to it from the first.' This observation lucidly describes a phenomenon that in the recent past has attracted much attention because it reflects some kind of learning in herbivorous insects, creatures that for a long time were considered to display only innate and simple stimulus–response behaviour patterns. Kirby and Spence's observation fell into oblivion until it was rediscovered and given a firm experimental basis by Jermy and colleagues.[68] These authors reared larvae of the corn earworm *Helicoverpa zea* on an artificial diet until the end of the fourth instar. After moulting to the fifth instar the insects were divided into four groups and were then fed during the whole fifth instar with one of the following foods: artificial diet, *Pelargonium hortorum* (geranium), *Taraxacum officinale* (dandelion), or *Brassica oleracea* (cabbage). When freshly moulted sixth-instar larvae were given a choice of leaf discs from the three plants (Fig. 8.7), it appeared that in each group of larvae preference had increased for the plant species they had fed upon during the fifth instar compared with the two other plants. Larvae that had no experience with any plant at all, because they were reared on an artificial diet ('naive' larvae), showed a pattern of preference that differed significantly from that of the other three groups (Fig. 8.8). This effect of previous experience on food-plant choice has been termed 'induced preference'.[68] Its nature and function are largely unknown and it does not fit into the usual categories of learning.

Insects reared on an artificial diet lacking any characteristic host-plant chemical seem to maintain the naivety of the newborn. They readily accept every host-plant species offered and even some non-hosts. Within 1–2 days on the plant food, however, their indifference is lost and an affinity for the plant experienced is established.[110] Food preferences in newly hatched larvae likewise develop with time. After 1–2 days of feeding on the plants on which they were born, larvae reject other foods (Fig. 8.9). Preference development based on dietary experience is probably caused by the taste of some plant constituents, which then serve as signals of suitability or unsuitability.[103,105]

In these examples of young larvae becoming committed to one of their host plant species there is a striking similarity with imprinting in young vertebrate animals. The term *imprinting* was originally coined for a form of irreversible learning that occurs during a very brief critical period, very early in an animal's life. The fact that food preference induction in insects may occur also later in larval life and, in addition, may be modified by new experiences, are reasons *not* to use the term imprinting in the context of preference induction.

The minimal duration of feeding needed for induction was demonstrated to be 4 h with larvae of *Pieris brassicae* feeding on *Tropaeolum majus*.[83] The persistence of an induced preference varies

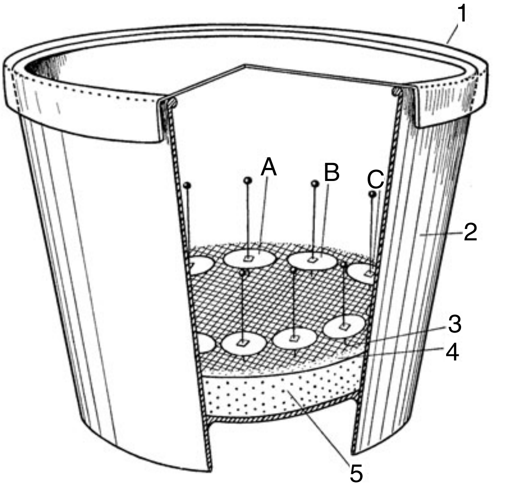

Figure 8.7 Arrangement for preference tests. A, B, C = leaf discs of three different plant species, mounted on pins; 1, cover of Petri dish; 2, paper food container cup; 3, wire screen; 4, moist filter paper; 5, paraffin layer. (From Jermy *et al.*, 1968.)[68]

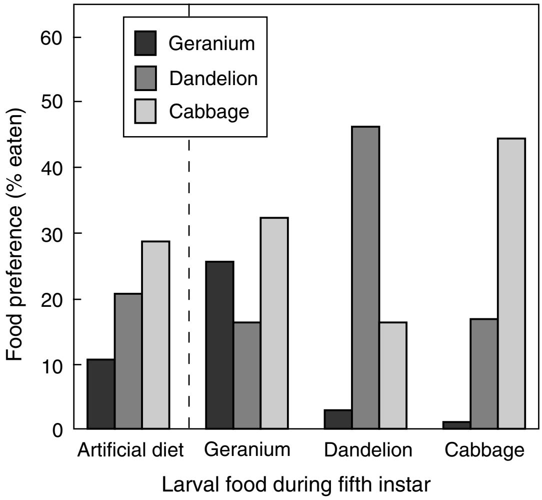

Figure 8.8 Food preference of sixth-instar larvae of *Helicoverpa zea* reared on artificial diet until the end of fourth instar and then fed during the fifth instar on artificial diet, geranium, dandelion, or cabbage. (Redrawn from Jermy *et al.*, 1968.)[68]

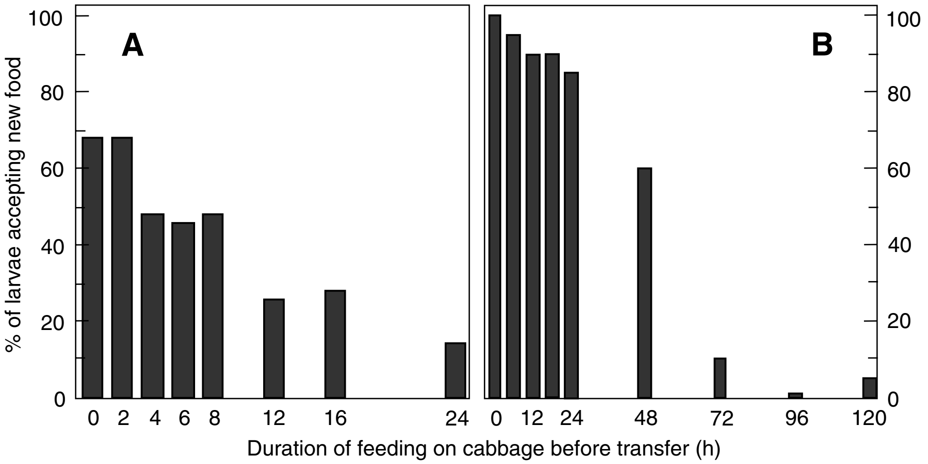

Figure 8.9 (**A**) Change in acceptance of nasturtium by first-instar larvae of *Pieris rapae* as a function of duration of feeding experience on cabbage plants. (Modified from Renwick and Huang, 1995.)[104] (**B**) Change in acceptance of artificial diet by first-instar larvae of *P. brassicae* as a function of duration of feeding experience on cabbage plants. (Modified from Schoonhoven, 1977.)[112] Note the difference in scale of the *X*-axes.

considerably among insect species and depends also on the plant species on which the insect gained experience. For example, as shown in Figure 8.8, induced preference for the plants in corn earworm larvae persisted through the moult from the fifth to the sixth instar. In another experiment with larvae of the same species an induced preference appeared to persist through two moults and the entire instar between these moults, during which the larvae were fed an artificial diet lacking any specific substance from their host plants.[68]

Induced preference is in some cases very rigid. Darwin[37] cites the observation made by M. Michely: 'The caterpillars of *Bombyx hesperus* [= *Rothschildia hesperus*] feed in a state of nature on the leaves of the *Café diable* [= *Hamamelis virginiana*], but, after having been reared on the *Ailanthus*, they would not touch the *Café diable*, and actually died of hunger'. When *Pieris brassicae* larvae were reared on cabbage and the young fifth-instar larvae transferred to another host plant, *Tropaeolum majus*, the larvae also refused to feed and as a consequence died from starvation.[83] This same rigidity of induction was observed in some other lepidopterans.[115] Such an extreme form of preference induction has, referring to the lavish meals of the Roman general Lucullus, been called the 'starving-to-death-at-Lucullian-banquets' phenomenon.[67]

Induced preference has been reported to occur in several dozen insect species belonging to six different orders: Orthoptera, Phasmida, Heteroptera, Homoptera, Coleoptera, and Lepidoptera. The broad distribution of this phenomenon indicates that we are dealing with a generally occurring type of behavioural change. The most striking examples, however, are known from lepidopterous larvae.[133] That is not to say, however, that the phenomenon is universal among herbivores. It could not be found in several lepidopteran or acridid species.[62,67]

A multiplicity of mechanisms has been suggested to produce induced food preferences and related changes,[17] but the physiological processes involved are still largely beyond our grasp. As for the neural background of preference induction, it is not known what role the peripheral organs (receptors) or the central nervous system play. In some cases the sensitivity of the chemoreceptors increases for host-specific compounds when the insects are reared on that plant. Increased chemoreceptor sensitivity to specific plant compounds in insects after exposure to a host plant characterized by particular chemicals is exemplified by *Spodoptera littoralis* larvae. When reared on cabbage they show a higher sensitivity to glucosinolates than when grown on an artificial diet lacking such

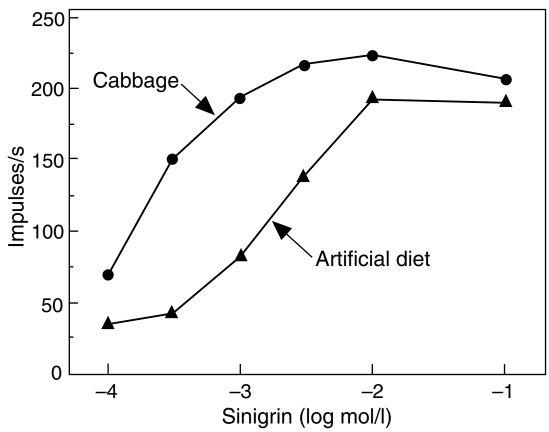

Figure 8.10 Neural responses (impulses/s) of the medial taste hair (sensillum styloconicum) on the maxillae of fifth-instar larvae of *Spodoptera littoralis* when stimulated by a glucosinolate (sinigrin) at various concentrations. The insects were reared on cabbage leaves or on an artificial bean-based diet. Cabbage-reared caterpillars showed a significantly higher sensitivity to the glucosinolate than those reared on the artificial diet. (From Schoonhoven *et al.*, 1987.)[114]

compounds (Fig. 8.10).[114] A similar observation has recently been reported for another caterpillar species. Larvae of *Estigmene acrea*, when grown on a mixed plant diet including species that contain pyrrolizidine alkaloids (PAs), showed an increase in responsiveness of two PA-sensitive receptor cells to these compounds. When the caterpillars were reared for several generations on synthetic diets lacking PAs, a continuous decline in responsiveness was observed, that could be reversed, however, by experience with a diet containing pure PAs.[30] Therefore, it is likely that food plants that have been eaten before produce stronger neural stimulation than novel food plants.[39]

The existence of the 'peripheral learning' phenomenon in insect chemosensory systems, although an important attribute of the nervous system, does not imply any lesser role for the central nervous system: primacy of preference behaviour still resides in the CNS.

The phenomenon of preference induction clearly proves that insects are able to discriminate not only between host- and non-host-plant species, but also between initially equally acceptable host-plant species. Thus, herbivorous insects are able to perceive a very detailed 'chemosensory profile',[38] or a 'chemical Gestalt',[76] for each host-plant species. This notion emphasizes the subtle complexity of stimuli involved in host recognition, based on both taste and olfaction.

Whereas natural stimuli guiding the insect's feeding behaviour are quite complex, it is sometimes possible to identify single compounds that play a major role in food preference induction. For example, indioside D, a steroidal glycoside present in solanaceous foliage, is involved in preference induction in the tobacco hornworm.[39] Volatile compounds may also serve as cues for recognizing the food on which preference induction occurred, as has been seen in caterpillars of *Manduca sexta* (Fig. 8.11)[108] and *Spodoptera littoralis*.[28]

8.6.2 Associative changes

(a) Food aversion learning

Aversion learning in herbivorous insects is, according to Dethier's definition,[41] an acquired aversion for a plant that had induced temporary malaise. This phenomenon, well known in vertebrates, including humans,[97] was first studied in larvae of the arctiid moth *Diacrisia virginica*. These caterpillars, while moving through short vegetation and taking bites from a number of plant individuals and species, forage as true polyphages. When offered leaves of *Petunia hybrida*, a plant not found in their natural habitat, they greedily consumed it. Ingestion for more than 24 h, however, produced malaise (regurgitation, decrease in activity, locomotor ataxia, mild convulsion, and bloating in the thoracic region). When such larvae, after recovery, were placed in a field arena with other plant species, they avoided *Petunia*, in contrast to unexperienced (naive) larvae (Fig. 8.12).[42] The survival value of this type of learning is self-evident, but it is still not known how common aversion learning is in insects. So far it has been observed in only a few caterpillar species and in one or two acridids.[12] Possibly it is more common in polyphagous species than in specialists.[43] A further example of experience-induced food rejection was found in oligophagous *Locusta migratoria* adults. At the first encounter this insect may begin to palpate and then take a bite before it rejects a non-host plant. At subsequent encounters with that particular

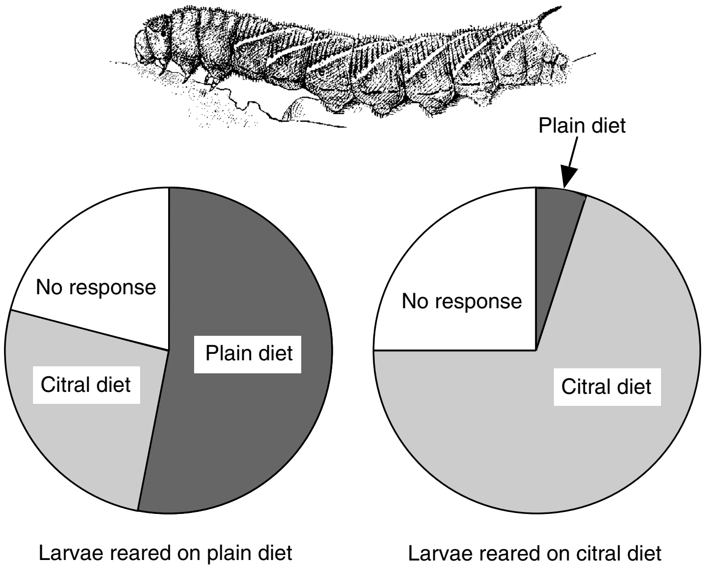

Figure 8.11 Olfactory orientation of fifth-instar larvae of *Manduca sexta* reared on artificial diet with or without citral. The two diets were presented in a choice test at short distances from the experimental insect. Responses are shown as percentages of larvae moving towards either the citral diet or the plain diet. (Data from Saxena and Schoonhoven, 1978.)[108]

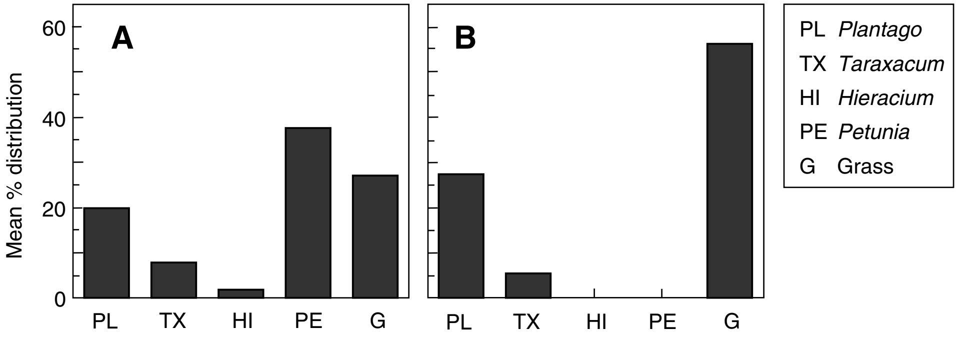

Figure 8.12 Mean percentage distribution of *Diacrisia virginica* caterpillars in field plots over a 2-day period. (**A**) Naive caterpillars. (**B**) Distribution after recovery of caterpillars fed exclusively on *Petunia* for 48 h. (Data from Dethier, 1988.)[42]

plant species, palpation suffices only to decide rejection.[21] In this case learning takes place by associating the information provided by the palpal chemoreceptors from the leaf surface with the information received when taking a bite from the distasteful ('punishment') leaf contents.[20] Such a learned response did not wane in another acridid, *Schistocerca americana*, even when the insect had been without food for over 2 h.[29]

(b) Dietary self-selection (mixed diet)

Although most herbivorous insect are highly specific in host-plant choice and induction of feeding preference represents a further temporary or permanent restriction in food plant range (see Section 8.6.1b), in some species an opposite behaviour has been observed—a craving for a mixed diet. This is expressed by frequent shifts between different foods. This phenomenon was first described for the polyphagous larvae of *Malacosoma castrensis*. When caterpillars were confined to one of the food-plant species on which they are found in nature, growth was retarded and the mortality rate was high. When caged with a number of plant species, the caterpillars were seen often to switch between plants and their survival rate was much higher than on a single-plant diet. Likewise, *Arctia caja* caterpillars showed such voluntary food switches and, as a result, grew better.[86] The most frequent switching among food plants is found in grasshoppers and locusts. Several species are known to die, or at least to show reduced survival and fecundity rates, when restricted to a single food-plant species.[6,72] Field observations showed that the grasshopper *Taeniopoda eques*, which lives in south-western parts of North America in habitats containing a great diversity of plants, may ingest up to eight different plant species within one meal.[18]

Two physiological advantages of feeding on mixed diets rather than on a single plant species have been suggested. Food-mixing could (1) result in a better matching of the optimal intake of nutrient composition and/or (2) prevent the intake of excessive doses of toxic secondary metabolites from particular host-plant species. It may be difficult to distinguish between the two hypotheses, but it has been argued that the two physiological explanations may be discriminated on the basis of consumption and performance parameters. If the insect is striving to optimize its intake of critical complementary nutrients, it will grow faster on a mixed diet without necessarily consuming more. However, if the insect seeks to dilute several different plant toxins by diet mixing, then it will ingest larger amounts than on a single plant and, as a result, will show increased performance.[19]

In grasshoppers three mechanisms causing dietary mixing have been recognized so far. The importance of each of them is probably related to both the feeding behaviour of the species and the habitat in which the species occurs.[15] One of the possible mechanisms is *food aversion learning*. For example, when the polyphagous grasshopper *Schistocerca americana* is fed only spinach, it takes progressively shorter meals and finally rejects spinach completely. As no changes in the chemoreceptor system were found, it was assumed that the insect associated the aversive consequences (malaise) of eating spinach with prior experience of its taste.[79] At the same time, alternative flavours became more attractive, suggesting that learned aversion may be accompanied by a predilection for novelty (*neophilia*). Learning predominates in polyphagous grasshoppers that live in simple habitats where few plant species are available and the insect may therefore be forced to feed for a longer time on a single plant species.[15]

Positive associative learning can occur by the phenomenon of 'specific hunger'. Locusts that previously experienced a high-protein diet that contained a specific odorous compound were significantly attracted to that odour when deprived of protein for some time.[123]

A second possible mechanism involves the *modulation of chemoreceptors*. The sensitivity of the palpal receptors to amino acids or to sugars may change in response to haemolymph nutrient composition. Feeding on a diet low in protein results in a low concentration of amino acids in the haemolymph, and this in turn results in an increase in the sensitivity of the chemoreceptors to amino acids in the food (see Section 5.3.3a). Consequently, the insect switches to a new diet rich in protein (i.e. containing more amino acids). The same mechanism operates with respect to carbohydrate content of the food: if the diet is low in carbohydrates, the sugar level of the haemolymph decreases, which in turn decreases in some unknown way the sensitivity threshold of the receptors to sugar.[1] Similar variations in neural activity corresponding to the pattern of protein and carbohydrate ingestion occur in caterpillars such as the polyphagous noctuid *Spodoptera littoralis*.[122] This chemoreceptor modulation mechanism occurs also in specialist species, such as the tobacco hornworm and the migratory locust *Locusta migratoria*, which feeds on grass species. Grasses are more difficult to distinguish from one another than dicots, because the latter group of plants shows a higher degree of diversification in secondary plant substances.[15] Interestingly, in this insect species the number of chemosensilla present on the maxillary palps may change during development in response to diet characteristics. Such changes are biologically relevant, because they are reflected in changes in feeding behaviour.[88]

A third mechanism is *arousal by novelty*. The grasshopper *Taeniopoda eques*, for example, is a 'compulsive' switcher, as newly detected odour (i.e. chemical novelty) provides an arousal stimulus leading to feeding. For this insect 'variety is the spice of life'.[18] This mechanism is important in grasshoppers that are very mobile and live in complex habitats with numerous plant species in close proximity.[15] This case shows that neophilia occurs not only in association with aversion learning, as exemplified above by *Schistocerca americana*, but also as an apparently automatic change in preference.

Members of other insect orders also enjoy the benefits of a mixed diet. The xylophagous leafhopper *Homalodisca coagulata*, for example, shows discrete shifts in host usage during its long lifespan, thereby increasing survival.[27] Likewise, two oligophagous grass-feeding heteropterans showed much higher survival rates when grown on a mixture of four host-plant species compared with monocultures of one host plant.[44]

(c) Experience-induced changes in oviposition behaviour

Egg-laying females may gain experience when they oviposit on a specific substrate for the first time. This experience can influence subsequent choices of oviposition substrates. When apple maggot flies (*Rhagoletis pomonella*) were trained by letting them oviposit on apples and were then offered fruits of another host plant, *Crataegus mollis*, these were not accepted. Conversely, flies trained on *C. mollis* fruits rejected apple for oviposition.[95] This behaviour is a form of associative learning, as the flies associate the size and surface chemistry of the fruits they encountered first with the successful completion of egg-laying behaviour. The flies can discriminate not only different host species but even among different apple cultivars, as they develop a preference for the cultivar on which their first oviposition experience was gained.[94]

Several butterflies use visual cues in oviposition site selection. For example, *Battus philenor* can visually discriminate between two *Aristolochia* host species based on their different leaf shapes. Contact chemosensory experience with a host plant, even without an egg being laid, reinforces recognition of the specific leaf shape.[91] Females of *Heliconius* butterfly species, at the first encounter with a host plant, associate the shape of its leaves with their chemistry and afterwards search for the same leaf shape.[56] When the host plants are recognized by leaf shape, the females supposedly use a 'search image'.[98] It seems likely that in many of these cases this neurally stored image also includes volatile plant cues.[78]

Laboratory experiments have shown that ovipositing females of *Pieris rapae*[139] and *Helicoverpa armigera*, a nocturnal lepidopteran, also show changes in both pre- and post-alighting host selection resulting from previous experience.[34] All in all, adult learning in oviposition behaviour is well established as a fairly common phenomenon.[92] Nevertheless, just as the phenomenon of induced food preferences cannot be detected in some insect species, not every species develops a preference for a particular host plant as a result of previous experiences. Thus, females of *Papilio glaucus*, the most polyphagous swallowtail butterfly known, failed to show in a multiple-choice test any oviposition preference induction after a 2-day prior exposure to one of its host species.[116]

Host finding and host recognition by females searching for oviposition sites entails more complex information than during food selection because, in flying insects, as well as the whole process of navigation, visual and chemical stimuli (olfactory and gustatory) are operating. This, together with the fact that in nature flying insects may cover large distances between two successive egg depositions, makes an analysis of the role of experience in oviposition behaviour under natural conditions methodologically very difficult.

8.7 Pre- and early-adult experience

Some entomologists suggested long ago that larval experience gained on a host-plant species increased the preference for that plant as a feeding and/or oviposition substrate in the adult stage. The American entomologist A.D. Hopkins was among the first to publish some evidence for this phenomenon and pointed to its relevance for evolutionary changes in host-plant preference. The 'Hopkins host-selection principle' states in the wording by its proposer, that, 'an insect species which breeds in two or more hosts will prefer to continue to breed in the host to which it has become adapted'.[33] In view of its importance for evolutionary processes, many authors have sought a transfer of larval feeding experience to the adult stage, but generally with negative results.[7,133] (This does not necessarily mean that Hopkins' inference was wrong. Experiments that might confirm his hypothesis are difficult to repeat, because the insect species used in his laboratory (e.g. cerambycids) are experimentally difficult to handle and have a long life cycle.[33])

Jaenike[65] proposed a 'neo-Hopkins host-selection principle', which states that the exposure of insects early in their adult stadium to a particular type of host will often increase their preference for that host. Such early adult experience has been demonstrated in *Drosophila*:[137] when fully developed larvae and/or newly formed puparia were thoroughly cleaned from the remainder of the larval culture medium by washing, the adults were less attracted to that food than adults developing from the unwashed larvae and/or puparia. Following this line of thought, Corbet[32] proposed the 'chemical legacy hypothesis', emphasizing that, even if the puparia are washed, some chemical traces originating from the larval food may persist in the haemolymph of the insect or on the outside of the pupa, and modify the perception and behaviour of the emerging adult.

Although it has not been proved experimentally, similar early-adult experience of the food plant may be present in insects that emerge as adults from that plant part where they developed as larvae, such as species developing in stalks and wood, in fruits and seeds, in mines, galls, etc.

Evidence that learning actually takes place in the immature and is transferred to the adult is scarce. In an interesting experiment *Drosophila* larvae were trained to avoid electric shocks by a Pavlovian conditioning procedure using a specific odour. The odour avoidance behaviour was maintained through metamorphosis in spite of the extensive reorganization of the nervous system during metamorphosis.[8,140] This experiment shows that, in principle, memory transfer from the larval stage to the adult is possible. In the case of the Mexican bean beetle (*Epilachna varivestis*) habituation to a feeding deterrent during the larval stage also appeared to be transferred over metamorphosis to the adult stage.[3] This study, as well as three others using *Plutella xylostella*, *Trichoplusia ni*,[2] *Spodoptera littoralis*,[4] and paper wasps,[101] suggests that memory storage occurs during metamorphosis. Memory transfer in these cases might be explained by Corbet's chemical legacy hypothesis, but without further evidence storage of information in the central nervous system cannot be ruled out.

8.8 Adaptive significance of experience-induced changes in host preference

A common flaw of studies on experience-induced changes in host preference behaviour is that they are based, almost without exception, on laboratory experiments alone. Therefore we do not know to what extent the results can be extrapolated to nature. Nevertheless, supposing that the above-discussed phenomena also exist under natural conditions, it is logical to ask: what is the adaptive value of such changes?

Habituation to deterrents occurred in experiments using single compounds at concentrations that, in a no-choice situation, did not prevent feeding totally, whereas habituation did not occur with strong deterrents or with non-host plants. The adaptive significance of habituation to feeding-inhibitory stimuli could thus lie in the possibility of an insect behaviourally overcoming slight increases in the concentration of deterrent chemicals in its host plants resulting from, for instance, environmental factors. It could also allow insects to exploit marginally acceptable, initially deterrent plants, provided they are not toxic, in a situation where no better plants are available.

The adaptive advantage of *food aversion learning* is self-evident: it prevents the consumption of deleterious quantities of poisonous or nutritionally inadequate plants. Polyphagous insects are especially prone to ingest poisonous plants. Therefore, natural selection has promoted evolution of the capacity for aversion learning in these insects, whereas specialist feeders are prevented from feeding on physiologically unsuitable plants by their finickiness about host-plant choice. These species may therefore lack the capacity for aversion learning,[43] but our knowledge on this point is scanty.

The adaptive advantage of *induced feeding preference* is less evident. It has been assumed that it reflects an adaptation of insects in which frequent changes of food type decrease the efficiency of food utilization.[60,71,115] If forced to switch to a novel host, several insect species (but not all—see, for instance, Pescador[93]) incurred a high physiological cost, evidenced by reduced growth and other fitness parameters (Table 8.3). It seems that insects become physiologically or biochemically adapted to the host plant on which they have fed for some time. The role of induced preference behaviour then may be to prevent a change of food in a vegetation where various host plants may occur, for instance with intertwined shoots or leaves. Although this hypothesis is intuitively attractive, the adaptive advantage of some insects starving to death amidst suitable though unfamiliar host plants is difficult to comprehend.[67] Furthermore, a strong induced feeding preference, which forces insects to search for familiar food even in the presence of suitable alternative hosts, may slow down larval development and thus prolong exposure to predators and parasites. However, when, for instance, a larva falls off its host plant, an increased sensitivity to host odour may help it to refind the host. Induction may also increase the intensity of normal food intake by heightening arousal and minimizing interruptions in feeding.

Induced oviposition preference is thought to enhance foraging efficiency within resource patches[92] and to reduce the likelihood that a female entering a patch of rare or unsuitable hosts will stay there and lose time in fruitless searching.[96] The finding that specialists need less time to recognize a host plant than generalists[66] also suggests that learning is an advantageous feature in generalist species. Furthermore, females depositing eggs on more abundant host-plant species would ensure a higher survival rate of their progeny.[99] More indirectly, learning in ovipositing females may favour larval performance in cases where a larva needs more than one plant in order to complete development. When its mother concentrates on the most abundant host-plant species, the chances that the larva must switch to another plant species are lowest.[35] However, in this case also one cannot help wondering what advantage might arise from behaviour that results in oviposition on one host-plant species in a habitat but prevents oviposition on several other suitable host species co-occurring with the host chosen originally.[70,99]

Table 8.3 Performance of *Colias philodice* larvae on two host plant species, *Medicago sativa* and *Melilotus alba*, and its modification by food-plant switch (modified from Karowe 1989)[71]

Food plants (rearing/tested)	RGR	Instar duration (h)	Total food consumed (mg dry wt)	Pupal weight (mg dry wt)
sativa/sativa	0.26	104	125	29
sativa/alba	0.08	150	81	14
alba/alba	0.31	124	162	34
alba/sativa	0.11	141	89	16

Note: Fifth instar larvae were tested for various performance parameters when fed their rearing plant or an alternative food plant. Relative growth rate (RGR) = mg of dry matter of growth per mg of dry bodyweight per day.

It should be considered that both induced feeding and induced oviposition preferences represent a temporary or permanent restriction of the innate host specificity of a herbivorous insect population or of an individual. It is most probable that not all induced changes of host preferences are adaptive. Cases like the 'starving-to-death-at-Lucullian-banquets' phenomenon or the avoidance of otherwise suitable oviposition substrates may indicate the limited ability of the insect's nervous system to switch from a behaviour just learned to another, innate, behaviour, or to learn a new one. Thus, induction of preference might be a kind of (chemical or visual) 'tunnel vision' due to some basic inertia in the insect's nervous system.[14,133]

The still incomplete picture of the phenomena of habituation and preference induction shows, as a general trend, that these processes are more pronounced in generalists than in specialists.[16] Whether or not this is wholly or partly related to limitations of the neural system remains to be elucidated.

In conclusion, although the adaptive value of some forms of associative learning seems obvious,[12,52] as yet no fully satisfactory answer can be given to the question of adaptive advantage of all types of experience-induced behaviour.[67]

8.9 Conclusions

This chapter aimed to demonstrate the existence of significant amounts of variation at the intraspecific level in feeding and oviposition behaviour for both host preference, or degree of specialization to particular host plants, and host suitability. The study of such variation contributes greatly to the understanding of resource use and other ecological processes, and of adaptations to stressed environments such as those caused by agricultural practices.[40,73]

Analysis of the variation or plasticity of an insect's behaviour is complicated by a simultaneous variation on the part of the plant. Its nutritional quality varies over time, and its genetic constitution varies with place and time. Additionally, one can surmise that the nutritional requirements of insects change during ontogeny by virtue of shifting demands as a function of growth, reproduction, and migration, processes that may affect an insect's food preference.

Some variations in food specialization within and among populations are also caused by differences in experience or maternal effects, whereas others reflect differences in genetic make-up. The fact that induced feeding preferences have been observed in all major herbivorous insect taxa suggests that it represents a basic phenomenon. At the same time, the fact that several species seem to lack this capacity entirely makes its function the more mysterious.

The finding that the memory of previous experiences is not located solely in the central nervous system, but may also be (partly) stored in chemoreceptor neurons, provides evidence for the notion that all cells and organs are in some way or another modulated by previous influences. The memory located in neurons is complementary to memory shown by, for instance, the digestive system, where adaptation of detoxifying enzymes to different types of food occurs (see Section 5.3.5).

The various points discussed in this chapter have clearly shown that the relationship between an insect species and its host plants is not as absolute and fixed as might be thought at first sight. These relationships, rather, show a considerable flexibility, which is on the one hand essential to maintain them and on the other a prerequisite for new evolutionary developments. At the same time, to use Jermy's words, 'induced feeding and oviposition preferences are nothing other than a further restriction in the overall finickiness of phytophagous insect species at the population and/or individual levels.'[67]

In conclusion, an insect that possesses the average of all behavioural (and other biological) features does not exist. Each individual is a unique combination of inherited and acquired traits.

8.10 References

1. Abisgold, J.D. and Simpson, S.J. (1988). The effects of dietary protein levels and haemolymph composition on the sensitivity of the maxillary palp chemoreceptors of locusts. *Journal of Experimental Biology*, **135**, 215–29.
2. Akhtar, Y. and Isman, M.B. (2003). Larval exposure to oviposition deterrents alters subsequent oviposition behavior in generalist, *Trichoplusia ni* and specialist, *Plutella xylostella* moths. *Journal of Chemical Ecology*, **29**, 1853–70.
3. Akhtar, Y. and Isman, M.B. (2004). Feeding responses of specialist herbivores to plant extracts and pure allelochemicals: effects of prolonged exposure. *Entomologia Experimentalis et Applicata*, **111**, 201–8.
4. Anderson, P., Hilker, M., and Löfqvist, J. (1995). Larval diet influence on oviposition behaviour in *Spodoptera littoralis*. *Entomologia Experimentalis et Applicata*, **74**, 71–82.
5. Barbosa, P., Martinet, P., and Waldvogel, M. (1986). Development, fecundity and survival of the herbivore *Lymantria dispar* and the number of plant species in its diet. *Ecological Entomology*, **11**, 1–6.
6. Barnes, O.L. (1965). Further tests of the effects of food plants on the migratory grasshopper. *Journal of Economic Entomology*, **58**, 475–9.
7. Barron, A.B. (2001). The life and death of Hopkins' host-selection principle. *Journal of Insect Behavior*, **14**, 725–37.
8. Barron, A.B. and Corbet, S.A. (1999). Preimaginal conditioning in *Drosophila* revisited. *Animal Behaviour*, **58**, 621–8.
9. Baylis, M. and Pierce, N. (1993). The effects of ant mutualism on the foraging and diet of lycaenid caterpillars. In *Caterpillars. Ecological and evolutionary constraints on foraging* (ed. N.E. Stamp and T.M. Casey), pp. 404–21. Chapman & Hall, New York.
10. Beckerman, A.P., Uriarte, M., and Schmitz, O.J. (1997). Experimental evidence for a behavior-mediated trophic cascade in a terrestrial food chain. *Proceedings of the National Academy of Sciences of the USA*, **94**, 10735–8.

11. Bennett, A.F. (1987). Interindividual variability: an underutilized resource. In *New directions in ecological physiology* (ed. M.E. Feder, A.F. Bennett, W.W. Burggren, and R.B. Huey), pp. 147–69. Cambridge University Press, Cambridge.
12. Bernays, E.A. (1995). Effects of experience in host-plant selection. In *Chemical ecology* (ed. R. Cardé and W. Bell), pp. 47–64. Chapman & Hall, New York.
13. Bernays, E.A. (1995). Effects of experience on feeding. In *Regulatory mechanisms in insect feeding* (ed. R.F. Chapman and G. de Boer), pp. 297–306. Chapman & Hall, New York.
14. Bernays, E.A. (2001). Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annual Review of Entomology*, **46**, 703–27.
15. Bernays, E.A. and Bright, K.L. (1993). Mechanisms of dietary mixing in grasshoppers: a review. *Comparative Physiology and Biochemistry A*, **104**, 125–31.
16. Bernays, E.A. and Funk, D.J. (1999). Specialists make faster decisions than generalists: experiments with aphids. *Proceedings of the Royal Society, Series B*, **266**, 151–6.
17. Bernays, E.A. and Weiss, M.R. (1996). Induced food preferences in caterpillars: the need to identify mechanisms. *Entomologia Experimentalis et Applicata*, **78**, 1–8.
18. Bernays, E.A., Bright, K., Howard, J.J., and Raubenheimer, D. (1992). Variety is the spice of life: frequent switching between foods in the polyphagous grasshopper *Taeniopoda eques* Burmeister (Orthoptera: Acrididae). *Animal Behaviour*, **44**, 721–31.
19. Bernays, E.A., Bright, K.L., Gonzalez, N., and Angel, J. (1994). Dietary mixing in a generalist herbivore: tests of two hypotheses. *Ecology*, **75**, 1997–2006.
20. Blaney, W.M. and Simmonds, M.S.J. (1985). Food selection by locusts: the role of learning in selection behaviour. *Entomologia Experimentalis et Applicata*, **39**, 273–8.
21. Blaney, W.M. and Winstanley, C. (1982). Food selection behaviour in Locusta migratoria. In *Proceedings of the 5th International Symposium on Insect–Plant Relationships* (ed. J.H. Visser and A.K. Minks), pp. 365–6. Pudoc, Wageningen.
22. Bomford, M.K. and Isman, M.B. (1996). Desensitization of fifth instar *Spodoptera litura* (Lepidoptera: Noctuidae) to azadirachtin and neem. *Entomologia Experimentalis et Applicata*, **81**, 307–13.
23. Bongers, W. (1970). Aspects of host-plant relationships of the Colorado beetle. *Mededelingen Landbouwhogeschool Wageningen*, **70-10**, 1–77.
24. Bossart, J.L. (2003). Covariance of preference and performance on normal and novel hosts in a locally monophagous and locally polyphagous butterfly population. *Oecologia*, **135**, 477–86.
25. Bossart, J.L. and Scriber, J.M. (1999). Preference variation in the polyphagous tiger swallowtail butterfly (Lepidoptera: Papilionidae). *Environmental Entomology*, **28**, 628–37.
26. Boys, H.A. (1978). Food selection by *Oedaleus senegalensis* (Acrididae: Orthoptera) in grassland and millet fields. *Entomologia Experimentalis et Applicata*, **24**, 278–86.
27. Brodbeck, B.V., Andersen, P.C., and Mizell, R.F. (1995). Differential utilization of nutrients during development by the xylophagous leafhopper, *Homalodisca coagulata. Entomologia Experimentalis et Applicata*, **75**, 279–89.
28. Carlsson, M.A., Anderson, P., Hartlieb, E., and Hansson, B.S. (1999). Experience-dependent modification of orientational response to olfactory cues in larvae of *Spodoptera littoralis. Journal of Chemical Ecology*, **25**, 2445–54.
29. Chapman, R.F. and Sword, S. (1993). The importance of palpation in food selection by a polyphagous grasshopper (Orthoptera: Acrididae). *Journal of Insect Behavior*, **6**, 79–91.
30. Chapman, R.F., Bernays, E.A., Singer, M.S., and Hartmann, T. (2003). Experience influences gustatory responsiveness to pyrrolizidine alkaloids in the polyphagous caterpillar, *Estigmene acrea. Journal of Comparative Physiology A*, **189**, 833–41.
31. Chyb, S., Eichenseer, H., Hollister, B., Mullin, C.A., and Frazier, J.L. (1995). Identification of sensilla involved in taste mediation in adult western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *Journal of Chemical Ecology*, **21**, 313–29.
32. Corbet, S.A. (1985). Insect chemosensory responses: a chemical legacy hypothesis. *Ecological Entomology*, **10**, 143–53.
33. Craighead, F.C. (1921). Hopkins-host selection principle as related to certain cerambycid beetles. *Journal of Agricultural Research*, **22**, 189–220.
34. Cunningham, J.P., Mustapha, F.A., Jallow, M.F.A., Wright, D.J., and Zalucki, M.P. (1998). Learning in host selection in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Animal Behaviour*, **55**, 227–34.
35. Cunningham, J.P., West, S.A., and Zalucki, M.P. (2001). Host selection in phytophagous insects: a new explanation for learning in adults. *Oikos*, **95**, 537–43.
36. Cottrell, C.B. (1984). Aphytophagy in butterflies: its relationship to myrmecophily. *Zoological Journal of the Linnean Society*, **79**, 1–57.
37. Darwin, C. (1875). *The variation of animals and plants under domestication* (2nd edn). Methuen, London.

38. De Boer, G. and Hanson, F.E. (1984). Food plant selection and induction of feeding preference among host and non-host plants in larvae of the tobacco hornworm *Manduca sexta. Entomologia Experimentalis et Applicata*, **35**, 177–93.
39. Del Campo, M.L., Miles, C.I., Schroeder, F.C., Mueller, C., Booker, R., and Renwick, J.A.A. (2001). Host recognition by the tobacco hornworm is mediated by a host plant compound. *Nature*, **411**, 186–9.
40. Denno, R.F. and McClure, M.S. (1983). *Variable plants and herbivores in natural and managed systems*. Academic Press, San Diego.
41. Dethier, V.G. (1980). Food-aversion learning in two polyphagous caterpillars, *Diacrisia virginica* and *Estigmene congrua. Physiological Entomology*, **5**, 321–5.
42. Dethier, V.G. (1988). Induction and aversion-learning in polyphagous arctiid larvae (Lepidoptera) in an ecological setting. *Canandian Entomologist*, **120**, 125–31.
43. Dethier, V.G. and Yost, MT. (1979). Oligophagy and absence of food-aversion learning in tobacco hornworms *Manduca sexta. Physiological Entomology*, **4**, 125–30.
44. Di Giulio, M. and Edwards, P.J. (2003). The influence of host plant diversity and food quality on larval survival of plant feeding heteropteran bugs. *Ecological Entomology*, **28**, 51–7.
45. Dicke, M. and Grostal, P. (2001). Chemical detection of natural enemies by arthropods: an ecological perspective. *Annual Review of Ecology and Systematics*, **32**, 1–23.
46. Dickinson, J.L. (1992). Egg cannibalism by larvae and adults of the milkweed leaf beetle (*Labodomera clivicollis*, Coleoptera: Chrysomelidae). *Ecological Entomology*, **17**, 209–18.
47. Dixon, A.F.G. (1973). *Biology of aphids*. Edward Arnold, London.
48. Dixon, A.F.G. (1985). *Aphid ecology*. Blackie, Glasgow.
49. Dres, M. and Mallet, J. (2002). Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society of London*, **B357**, 471–92.
50. Drosopoulos, S. (1977). Biosystematic studies on the Muellerianella complex (Dephacidae, Homoptera, Auchenorrhyncha). *Mededelingen Landbouwhogeschool Wageningen*, **77-14**, 1–133.
51. Du, Y.-J., van Loon, J.J.A., and Renwick, J.A.A. (1995). Contact chemoreception of oviposition-stimulating glucosinolates and an oviposition-deterrent cardenolide in two subspecies of *Pieris napi. Physiological Entomology*, **20**, 164–74.
52. Dukas, R. and Bernays, E.A. (2000). Learning promotes growth rate in grasshoppers. *Proceedings of the National Academy of Sciences of the USA*, **97**, 2637–40.
53. Fox, L. (1975). Cannibalism in natural populations. *Annual Review of Ecology and Systematics*, **6**, 87–106.
54. Fox, L.R. and Morrow, P.A. (1981). Specialization: species property or local phenomenon? *Science*, **211**, 887–93.
55. Fritz, R.S. and Simms, E.L. (1992). *Plant resistance to herbivores and pathogens. Ecology, evolution and genetics*. University of Chicago Press, Chicago.
56. Gilbert, L.E. (1975). Ecological consequences of a coevolved mutualism between butterflies and plants. In *Coevolution of animals and plants* (ed. L.E. Gilbert and P.H. Raven), pp. 210–40. University of Texas Press, Austin.
57. Glendinning, J.I. (2002). How do herbivorous insects cope with noxious secondary plant compounds in their diet? *Entomologia Experimentalis et Applicata*, **104**, 15–25.
58. Glendinning, J.I., Domdom, S., and Long, E. (2001). Selective adaptation to noxious foods by a herbivorous insect. *Journal of Experimental Biology*, **204**, 3355–67.
59. Gould, F. (1993). The spatial scale of genetic variation in insect populations. In *Evolution of insect pests. Patterns of variation* (ed. K.C. Kim and B.A. McPheron), pp. 67–85. John Wiley, New York.
60. Grabstein, E.M. and Scriber, J.M. (1982). Host plant utilization by *Hyalophora cecropia* as affected by prior feeding experience. *Entomologia Experimentalis et Applicata*, **32**, 262–8.
61. Hare, J.D. and Kennedy, G.G. (1986). Genetic variation in plant–insect associations: survival of *Leptinotarsa decemlineata* populations on *Solanum carolinense. Evolution*, **40**, 1031–43.
62. Howard, G. and Bernays, E.A. (1994). Cited in Ref. 130.
63. Hsiao, T.H. (1985). Ecophysiological and genetic aspects of geographic variations of the Colorado potato beetle. In *Proceedings of the Symposium on the Colorado potato beetle, XVIIth International Congress of Entomology* (ed. D.N. Ferro and R.H. Voss), pp. 63–77. Massachusetts Agricultural Experiment Station, University of Massachusetts at Amherst.
64. Huang, X.P. and Renwick, J.A.A. (1995). Cross habituation to feeding deterrents and acceptance of a marginal host plant by *Pieris rapae* larvae. *Entomologia Experimentalis et Applicata*, **76**, 295–302.
65. Jaenike, J. (1983). Induction of host preference in *Drosophila melanogaster. Oecologia*, **58**, 320–5.
66. Janz, N. (2003). The cost of polyphagy: oviposition decision time vs error rate in a butterfly. *Oikos*, **100**, 493–6.

67. Jermy, T. (1987). The role of experience in the host selection of phytophagous insects. In *Perspectives in chemoreception and behavior* (ed. R.F. Chapman, E.A. Bernays, and J.G. Stoffolano), pp. 142–57. Springer, New York.
68. Jermy, T., Hanson, F.E., and Dethier. V.G. (1968). Induction of specific food preference in lepidopterous larvae. *Entomologia Experimentalis et Applicata*, **11**, 211–30.
69. Jermy, T., Bernays, E.A., and Szentesi, Á. (1982). The effect of repeated exposure to feeding deterrents on their acceptability to phytophagous insects. In *Proceedings of the 5th international symposium on insect–plant relationships* (ed. J.H. Visser and A.K. Minks), pp. 25–32. Pudoc, Wageningen.
70. Jones, R.E. and Ives, P.M. (1979.) The adaptiveness of searching and host selection behaviour in *Pieris rapae* (L.). *Australian Journal of Ecology*, **4**, 75–86.
71. Karowe, D.N. (1989). Facultative monophagy as a consequence of prior feeding experience: behavioral and physiological specialization in *Colias philodice* larvae. *Oecologia*, **78**, 106–11.
72. Kaufman, T. (1965). Biological studies of some Bavarian Acridoidea (Orthoptera) with special reference to their feeding habits. *Annals of the Entomological Society of America*, **58**, 791–800.
73. Kim, K.C. and McPheron, B.A. (1993). *Evolution of insect pests. Patterns of variation*. John Wiley, New York.
74. Kirby, W. and Spence, W. (1863). *An introduction to entomology* (7th edn). Longman, Green, Longman, Roberts & Green, London.
75. Klos, R. (1901). Zur Lebensgeschichte von *Tephroclystia virgaureata*. *Verhandlungen der Kaiserlichen-Königlichen Zoologisch-Botanischen Gesellschaft in Wien*, **51**, 785.
76. Kogan, M. (1977). The role of chemical factors in insect/plant relationships. *Proceedings of the 15th International Congress of Entomology*, Washington, DC, 211–27.
77. Kundu, R. and Dixon, A.F.G. (1995). Evolution of complex life cycles in aphids. *Journal of Animal Ecology*, **64**, 245–55.
78. Landolt, P.J. and Molina, O. (1996). Host-finding by cabbage looper moths (Lepidoptera: Noctuidae): learning of host odor upon contact with host foliage. *Journal of Insect Behavior*, **9**, 899–908.
79. Lee, J.C. and Bernays, E.A. (1988). Declining acceptability of food plant for a polyphagous grasshopper *Schistocerca americana*: the role of food aversion learning. *Physiological Entomology*, **13**, 291–301.
80. Leibrecht, B. C. and Askew, H. R. (1980). Habituation from a comparative perspective. In *Comparative psychology: an evolutionary analysis of animal behavior* (ed. M.R. Denny), pp. 208–229. John Wiley, New York.
81. Llewellyn, M. (1982). The energy economy of fluid-feeding herbivorous insects. In *Proceedings of the 5th international symposium on insect–plant relationships* (ed. J.H. Visser and A.K. Minks), pp. 243–51. Pudoc, Wageningen.
82. Lu, W. and Logan, P. (1994). Genetic variation in oviposition between and within populations of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Annals of the Entomological Society of America*, **87**, 634–40.
83. Ma, W.C. (1972). Dynamics of feeding responses in *Pieris brassicae* Linn. as a function of chemosensory input: a behavioural, ultrastructural and electrophysiological study. *Mededelingen Landbouwhogeschool Wageningen*, **72-11**, 1–162.
84. Mackenzie, A. and Dixon, A.F.G. (1990). Host alternation in aphids: constraint versus optimization. *American Naturalist*, **136**, 132–4.
85. Maier, C.T. (1985). Rosaceous hosts of *Phyllonorycter* species (Lepidoptera: Gracillariidae) in New England. *Annals of the Entomological Society of America*, **78**, 826–30.
86. Merz, E. (1959). Pflanzen und Raupen. Über einige Prinzipien der Futterwahl bei Großschmetterlingsraupen. *Biologisches Zentralblatt*, **78**, 152–88.
87. Moran, N.A. (1992). The evolution of aphid life cycles. *Annual Review of Entomology*, **37**, 321–48.
88. Opstad, R., Rogers, S.M., Behmer, S.T., and Simpson, S.J. (2004). Behavioural correlates of phenotypic plasticity in mouthpart chemoreceptor numbers in locusts. *Journal of Insect Physiology*, **50**, 725–36.
89. Panda, N. and Khush, G.S. (1995). *Host plant resistance to insects*. CAB International, Oxon.
90. Papaj, D. (1986a). An oviposition 'mistake' by *Battus philenor* L. (Papilionidae). *Journal of the Lepidopterists' Society*, **40**, 348–9.
91. Papaj, D. (1986b). Conditioning of leaf shape discrimination by chemical cues in the butterfly *Battus philenor*. *Animal Behaviour*, **34**, 1281–8.
92. Papaj, D.R. and Lewis, A.C. (1993). *Insect learning. Ecology and evolutionary perspectives*. Chapman & Hall, New York.
93. Pescador, A.R. (1993). The effects of a multispecies sequential diet on the growth and survival of tropical polyphagous caterpillars. *Entomologia Experimentalis et Applicata*, **67**, 15–24.
94. Prokopy, R.J. and Papaj, D.R. (1988). Learning of apple fruit biotypes by apple maggot flies. *Journal of Insect Behavior*, **1**, 67–74.
95. Prokopy, R.J., Averill, A.L., Cooley, S.S., and Roitberg, C.A. (1982). Associative learning of apple fruit biotypes by apple maggot flies. *Science*, **218**, 76–7.

96. Prokopy, R.J., Papaj, D.R., Cooley, S.S., and Kallet, C. (1986). On the nature of learning in oviposition site acceptance by apple maggot flies. *Animal Behaviour*, **34**, 98–107.
97. Provenza, F.D. and Cincotta, R.P. (1993). Foraging as a self-organizational learning process: accepting adaptability at the expense of predictability. In *Diet selection* (ed. R.N. Hughes), pp. 78–101. Blackwell, Oxford.
98. Rausher, M.D. (1978). Search image for leaf shape in a butterfly. *Science*, **200**, 1071–3.
99. Rausher, M.D. (1980). Host abundance, juvenile survival, and oviposition preference in *Battus philenor*. *Evolution*, **34**, 342–55.
100. Rausher, M.D. (1985). Variability for host preference in insect populations: mechanistic and evolutionary models. *Journal of Insect Physiology*, **31**, 873–89.
101. Rayor, L.S. and Munson, S. (2002). Larval feeding experience influences adult predator acceptance of chemically defended prey. *Entomologia Experimentalis et Applicata*, **104**, 193–201.
102. Reavy, D. and Lawton, J.H. (1991). Larval contribution to fitness in leaf-eating insects. In *Reproductive behaviour of insects: individuals and populations* (ed. W.J. Bailey and J. Ridsdill-Smith), pp. 293–329. Chapman & Hall, London.
103. Renwick, J.A.A. (2001). Variable diets and changing taste in plant–insect relationships. *Journal of Chemical Ecology*, **27**, 1063–76.
104. Renwick, J.A.A. and Huang, X.P. (1995). Rejection of host plant by larvae of cabbage butterfly: diet-dependent sensitivity to an antifeedant. *Journal of Chemical Ecology*, **21**, 465–75.
105. Renwick, J.A.A. and Lopez, K. (1999). Experience-based food consumption by larvae of *Pieris rapae*: addiction to glucosinolates? *Entomologia Experimentalis et Applicata*, **91**, 51–8.
106. Renwick, J.A.A., Zhang, W., Haribal, M., Attygalle, A.B., and Lopez, K.D. (2001). Dual chemical barriers protect a plant against larval stages of an insect. *Journal of Chemical Ecology*, **27**, 1575–83.
107. Sandlin, E.A. and Willig, M.R. (1993). Effects of age, sex, prior experience, and intraspecific food variation on diet composition of a tropical folivore (Phasmatodea: Phasmatidae). *Environmental Entomology*, **22**, 625–33.
108. Saxena, K.N. and Schoonhoven, L.M. (1978). Induction of orientation and feeding preferences in *Manduca sexta* larvae for an artificial diet containing citral. *Entomologia Experimentalis et Applicata*, **23**, 72–8.
109. Schalk, J.M., Kindler, S.D., and Manglitz, G.R. (1969). Temperature and the preference of the spotted alfalfa aphid for resistant and susceptible alfalfa plants (*Therioaphis maculata*: Hem., Hom., Aphididae). *Journal of Economic Entomology*, **62**, 1000–3.
110. Schoonhoven, L.M. (1967). Loss of host plant specificity by *Manduca sexta* after rearing on an artificial diet. *Entomologia Experimentalis et Applicata*, **10**, 270–2.
111. Schoonhoven, L.M. (1969). Sensitivity changes in some insect chemoreceptors and their effect on food selection behaviour. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Amsterdam*, **C72**, 491–8.
112. Schoonhoven, L.M. (1977). On the individuality of insect feeding behaviour. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Amsterdam*, **C80**, 341–50.
113. Schoonhoven, L.M. and van Loon, J.J.A. (2002). An inventory of taste in caterpillars: each species its own key. *Acta Zoologica Academiae Scientiarum Hungaricae*, **48** (Suppl. 1), 215–63.
114. Schoonhoven, L.M., Blaney, W.M., and Simmonds, M.S.J. (1987). Inconstancies of chemoreceptor sensitivities. In *Insects–plants. Proceedings of the 6th international symposium on insect–plant relationships* (ed. V. Labeyrie, G. Fabres, and D. Lachaise), pp. 141–5. Junk, Dordrecht.
115. Scriber, J.M. (1982). The behavioural and nutritional physiology of southern armyworm larvae as a function of plant species consumed in earlier instars. *Entomologia Experimentalis et Applicata*, **31**, 359–69.
116. Scriber, J.M. (1993). Absence of behavioral induction in multi-choice oviposition preference studies with a generalist butterfly species, *Papilio glaucus*. *Great Lakes Entomologist*, **28**, 81–95.
117. Scriber, J. M. (2002). Evolution of insect–plant relationships: chemical constraints, coadaptation, and concordance of insect/plant traits. *Entomologia Experimentalis et Applicata*, **104**, 217–35.
118. Scriber, J.M. and Lederhouse, R.C. (1992). The thermal environment as a resource dictating geographic patterns of feeding specialization of insect herbivores. In *Effects of resource distribution on animal–plant interactions* (ed. M.D. Hunter, T. Ohgushi, and P. Price), pp. 430–466. Academic Press, San Diego.
119. Scriber, J.M., Giebink, B.L., and Snider, D. (1991). Reciprocal latitudinal clines in oviposition behavior of *Papilio glaucus* and *P. canadensis* across the great Lakes hybrid zone: possible sex-linkage of oviposition preferences. *Oecologia*, **87**, 360–8.
120. Sergel, R. (1987). On the occurrence and ecology of the *Rhododendron*-leafhopper *Graphocephala fennahi*

Young 1977, in the western Palearctic region (Homoptera, Cicadellidae). *Anzeiger für Schädlungskunde, Pflanzenkrankheiten und Pflanzenschutz*, **60**, 134–6.

121. Shaposhnikov, G.C. (1987). Evolution of aphids in relation to evolution of plants. In *Aphids. Their biology, natural enemies and control*, Vol. A (ed. A.K. Minks and P. Harrewijn), pp. 409–414. Elsevier, Amsterdam.
122. Simmonds, M.S.J., Simpson, S.J., and Blaney, W.M. (1992). Dietary selection behaviour in *Spodoptera littoralis*: the effects of conditioning diet and conditioning period on neural responsiveness and selection behaviour. *Journal of Experimental Biology*, **162**, 73–90.
123. Simpson, S.J. and White, P. (1990). Associative learning and locust feeding: evidence for a 'learned hunger' for protein. *Animal Behaviour*, **40**, 506–13.
124. Simpson, C.L., Chyb, S., and Simpson, S.J. (1990). Changes in chemoreceptor sensitivity in relation to dietary selection by adult *Locusta migratoria. Entomologia Experimentalis et Applicata*, **56**, 259–68.
125. Singer, M.C. (1983a). Determinants of mutiple host use by a phytophagous insect population. *Evolution*, **37**, 389–403.
126. Singer, M.C. (1983b). Heritability of oviposition preference and its relationship to offspring performance within a single insect population. *Evolution*, **37**, 575–96.
127. Singer, M.C. and Lee, J.R. (2000). Discrimination within and between host species by a butterfly: implications for design of preference experiments. *Ecology Letters*, **3**, 101–5.
128. Singer, M.C. and Parmesan, C. (1993). Sources of variation in patterns of plant–insect association. *Nature*, **361**, 251–3.
129. Stockoff, B.A. (1993). Ontogenetic change in dietary selection for protein and lipid by gypsy moth larvae. *Journal of Insect Physiology*, **39**, 677–86.
130. Sword, G.A. and Chapman, R.F. (1994). Monophagy in a polyphagous grasshopper, *Schistocerca shoshone. Entomologia Experimentalis et Applicata*, **73**, 255–64.
131. Sword, G.A. and Dopman, E.B. (1999). Developmental specialization and geographic structure of host plant use in a polyphagous grasshopper, *Schistocerca emarginata* (= *lineata*) (Orthoptera, Acrididae). *Oecologia*, **120**, 437–45.
132. Szentesi, Á. and Bernays, E.A. (1984). A study of behavioural habituation to a feeding deterrent in nymphs of *Schistocerca gregaria. Physiological Entomology*, **9**, 329–40.
133. Szentesi, Á. and Jermy, T. (1990). The role of experience in host plant choice by phytophagous insects. In *Insect–plant interactions*, Vol. II (ed. E.A. Bernays), pp. 39–74. CRC Press, Boca Raton.
134. Szentesi, Á. and Jermy, T. (1993). A comparison of food-related behaviour between geographic populations of the Colorado potato beetle (Coleoptera, Chrysomelidae), on six solanaceous plants. *Entomologia Experimentalis et Applicata*, **66**, 283–93.
135. Tarpley, M.D., Breden, F., and Chippendale, G.M. (1993). Genetic control of geographic variation for cannibalism in the southwestern corn borer, *Diatraea grandiosella. Entomologia Experimentalis et Applicata*, **66**, 145–52.
136. Thompson, J.N. (1994). *The coevolutionary process*. University of Chicago Press, Chicago.
137. Thorpe, W.H. (1939). Further studies in preimaginal olfactory conditioning in insects. *Proceedings of the Royal Society*, **B127**, 424–33.
138. Tinbergen, L. (1960). The natural control of insects in pine woods. I. Factors influencing the intensity of predation by songbirds. *Archives Néerlandaises de Zoologie*, **13**, 265–336.
139. Traynier, R.M.M. (1986). Visual learning in assays of sinigrin solution as an oviposition releaser for the cabbage butterfly, *Pieris rapae. Entomologia Experimentalis et Applicata*, **40**, 25–33.
140. Tully, T., Cambiazo, V., and Kruse, L. (1994). Memory through metamorphosis in normal and mutant *Drosophila. Journal of Neuroscience*, **14**, 68–74.
141. Ueckert, D.N. and Hansen, R.M. (1971). Dietary overlap of grasshoppers on sandhill rangeland in northeastern Colorado. *Oecologia*, **8**, 276–95.
142. Van Loon, J.J.A. (1996). Chemosensory basis of feeding and oviposition behaviour in herbivorous insects: a glance at the periphery. *Entomologia Experimentalis et Applicata*, **80**, 7–13.
143. Via, S. (1999). Cannibalism facilitates the use of a novel environment in the flour beetle, *Tribolium castaneum. Heredity*, **82**, 267–75.
144. Zwölfer, H. (1970). Der 'Regionale Futterpflanzwechsel' bei phytophagen Insekten als evolutionäres Problem. *Zeitschrift für Angewandte Entomologie*, **65**, 233–7.
145. Zwölfer, H. (1988). Evolutionary and ecological relationships of the insect fauna of thistles. *Annual Review of Entomology*, **33**, 103–22.

CHAPTER 9

The endocrine system of herbivores listens to host-plant signals

Few places on earth provide conditions for the growth of plants and animals that remain constant throughout the year. Usually favourable seasons alternate with periods of low temperatures or drought. Plants tide themselves over these adverse periods by shedding their leaves, or the above-ground parts may die back completely. Annual species wait for the new growing season as dormant seeds in the soil. Insects may cease growth and reproduction, and enter diapause, a state of regulated inactivity. But even during the growing season plants change in their nutritive value (see Chapter 5), as well as the amounts of secondary plant substances they harbour (see Chapter 4). When a herbivorous insect strives optimally to exploit short-lived plants or particular developmental stages, an accurate synchronization of its life cycle with that of its host is of great adaptive value. Obviously, this is particularly true for food specialists and less important for generalist feeders. Synchronization is often well attained when both insects and plants respond to the same geophysical variables, notably changes in the photoperiod and related factors such as temperature and rainfall. Sensitivity to these cues allows insects to anticipate future environmental changes and to prepare for them through various inductive responses. A more refined synchronization of their phenology may be achieved when insects 'listen' to signals indicative of the developmental stage of their host plants. This ensures an accurate coupling of the insect's life cycle to that of its host, even when for some reason or other the host plant is slightly earlier or later than would be expected on the basis of, for instance, critical day length. Indeed, a great many insects monitor early indications of physiological changes in their host plant in order to set off the neuroendocrine mechanisms governing their development and reproduction at the appropriate time.

This chapter deals with plant factors, including olfactory, gustatory, and tactile stimuli, that may be used by herbivores to synchronize their life cycles as closely as possible to those of their hosts and, as far as they are known, the underlying physiological mechanisms.

9.1 Development

Developmental processes that may be governed by stimuli from the host plant include form determination (morphism) and induction of diapause.

9.1.1 Morphism

Aphids are well known for their environmentally determined polymorphism (or polyphenism). They often show different generations of seasonal forms

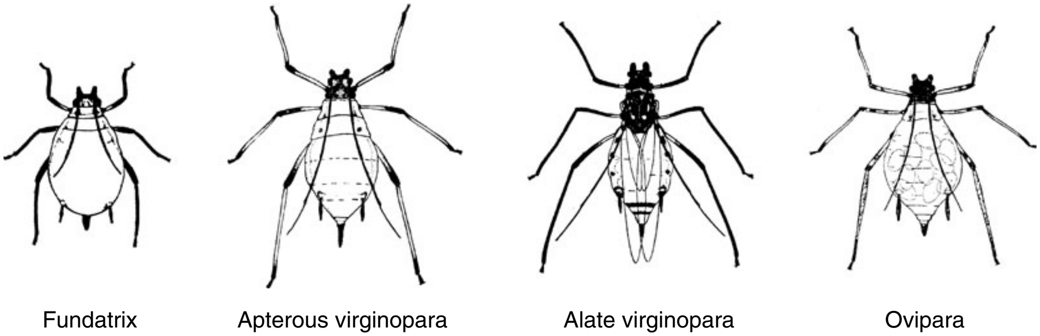

Figure 9.1 Female polymorphism in the vetch aphid *Megoura viciae*. (From Lees, 1961.)[23]

related to host alternation.[15] In many species the summer population can be either *apterous* (wingless) or *alate* (winged; see Fig. 9.1). The two morphs are both 'virginoparous' (they reproduce by parthenogenesis) but the alates have higher capacity to migrate to another host when the food plant becomes overexploited and its nutritional value starts to deteriorate. Nutritional quality appears to be an important factor influencing wing formation. In an experiment with pea aphids (*Acyrthosiphon pisum*) it was shown that the age of the host plant affected the occurrence of wing development. Likewise, stimuli arising from crowded conditions stimulate wing development in the offspring of apterous females, and the effects of host-plant age and crowding are additive (Fig. 9.2).

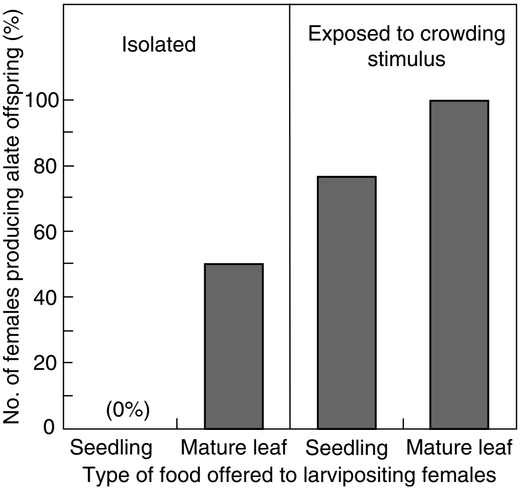

Figure 9.2 Role of host plant and crowding in the production of winged forms by apterous pea aphids. All aphids were reared in isolation on broad bean (*Vicia faba*) seedlings and as adults were either subjected to a crowding stimulus or were not. Larvipositing adults were kept on seedlings or on mature, fully expanded, green leaves. (Data from Sutherland, 1967.)[43]

The question that immediately arises concerns the nature of the factor or factors in the plant that induce the morphogenetic change to wing development. The answer to this question depends on which aphid–plant combination is studied, as many variations on the same physiological theme have been found. Often, the quality and amount of amino acids, sugars, and/or water content are the nutritional factors responsible for the formation of alates. Generally, a deterioration in the nutritional value of host saps evokes an irreversible formation of wings, thereby increasing migration capacity and the likelihood of finding better hosts. It is noteworthy that wing development does not necessarily indicate a nutritional deficiency but rather a response to some plant factor (or factors) that precedes the change in food quality. Complete development and vigorous growth of alate adults occurs on plants that are still fully adequate food for normal growth and reproduction.[15,18]

Cultures of aphids on an artificial diet (i.e. a chemically defined liquid food) provide an elegant method of determining the influence of dietary components on morph determination. When green peach aphids (*Myzus persicae*) were grown on such a diet it appeared that omission of vitamin C from the standard composition produced an increase in the relative proportion of alate offspring from 28% to 62%. However, when the concentration of amino acids was halved, the frequency of alates produced was reduced.[44] Apparently the developmental switch responsible for wing formation is affected in a number of insects by these specific nutrients. The fact that the switch is not activated in all individuals may be due to the signal being more complex under natural conditions.

In several aphid species the production of sexual forms in late summer or autumn is also influenced by host-plant factors. Hitherto unknown changes in ageing plants serve as signals to stimulate the production of sexuparae.[18] A striking example of a host effect on aphid morphs is found in *Eriosoma pyricola*, a species that lives on the roots of pear trees. During a limited period in late summer and early autumn alate sexuparous adults are produced, which emerge from the soil and then fly to elm trees, their primary host. The pear tree factor inducing the production of sexuparae is related to cessation of root growth, which in turn is regulated by photoperiod and temperature conditions affecting the above-ground tree parts. Direct effects of these environmental variables on the aphids could be excluded, but the effective plant factor has not been identified.[40]

What physiological mechanism links environmental triggers such as food quality to the developmental switch that governs the production of either alate or apterous adults? Juvenile hormone (JH) appears to play a key role in the process.[13] Larvae with low levels of JH produce winged females, whereas high JH levels, by affecting gene regulators, suppress wing formation. The question of how food quality affects the regulator of JH production, however, remains to be resolved.

Although the most dramatic examples of environmental polymorphism are found among aphids, the phenomenon also occurs in other insect taxa. For example, larvae of *Nemoria arizonaria*, a North American bivoltine moth species, show spectacular differences in appearance and behaviour between generations. Larvae of the spring generation feed on oak catkins and develop into highly cryptic mimics of catkins, showing a yellow rugose integument with reddish-brown stamen-like dots (Fig. 9.3A). Larvae of the summer brood feed on oak leaves and have a greenish-grey, less rugose, integument. Caterpillars of the latter form adopt the typical geometrid posture when disturbed (Fig. 9.3B). When raised on an artificial diet with (high) tannin concentrations reflecting natural food levels, caterpillars developed into twig morphs. Conversely, on tannin-free diets catkin morphs were produced. In this case also it is unclear how the plant stimulus (i.e. tannin level) elicits the appropriate developmental response.[12]

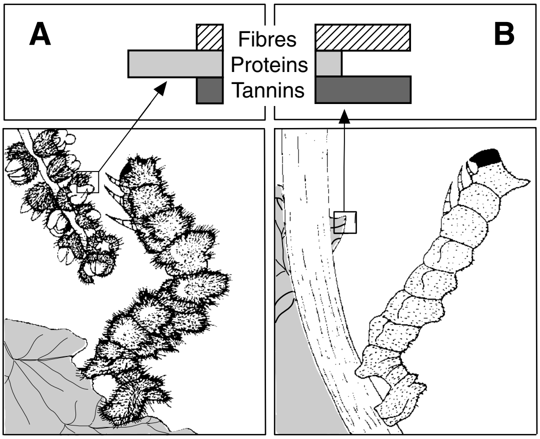

Figure 9.3 (**A**) Larvae of *Nemoria arizonaria* of the spring generation feeding on oak catkins and (**B**) larvae of the summer generation feeding on oak leaves develop into catkin morphs and twig morphs, respectively. Catkins contain less fibre and fewer polyphenols (tannins) but more unbound protein than leaves. (Redrawn from Dettner, 1989;[8] after Greene, 1989.[12])

Camouflage may go one step further and, in addition to visual features, involve chemical aspects. An example is provided by the geometrid *Biston robustum*. The body texture, colour, and shapes of this polyphagous caterpillar have been found to vary depending on the host-plant species that it fed on. Moreover, the caterpillar's cuticular wax components appear to vary depending on the food consumed, and resemble the surface chemicals of the twigs that the insect has perched on. The chemical similarities between the caterpillars and the twigs results from the digestion of host leaves, which indicates that this is a diet-induced adaptation. The mechanism involved in the morphological adaptation remains to be elucidated. The effectivity of this 'phytomimesis' was demonstrated by removing the caterpillars from the host plant on which they were raised and putting them on another host plant to which they had not yet been adapted. Whereas predatory ants negated the caterpillars totally on the first host plant, they did notice and subsequently attack the caterpillars on the new host.[1]

9.1.2 Diapause

Colorado potato beetles, after completion of metamorphosis, emerge from the soil and start feeding on the foliage of potato plants or a few related species. Under long-day photoperiods the beetles soon start to reproduce, but under short photoperiods they withdraw again into the soil and enter a period of diapause. When fed old rather than young potato leaves the beetles show a clear tendency to enter diapause, even under long-day conditions (Fig. 9.4).

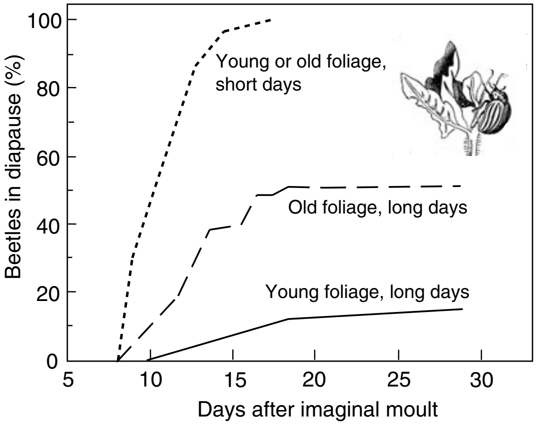

Figure 9.4 Effects of food quality (young versus old potato foliage) and day length (short versus long photoperiod) on diapause induction in adult Colorado potato beetles (*Leptinotarsa decemlineata*). (Redrawn from Danilevski, 1965.)[4]

This response cannot be attributed to a nutritive deficiency because, when the corpora allata of active insects (which produce JH) are implanted into diapausing recipients, normal activity and reproduction are resumed, even when fed ageing foliage.[9] The observation that wide differences in the incidence of diapause exist between field populations feeding on *Solanum dulcamara* (nightshade) and *S. tuberosum* (potato) likewise indicates that plant factors contribute to diapause induction. The adaptive significance of an earlier start of diapause in insects living on nightshade compared with potato is probably related to the fact that insects on late-season nightshade are confronted with very high levels of glycoalkaloids, causing high mortality rates.[14]

Once inside a herbivore's body, even parasitoids may begin diapause in response to age-related quality changes in the food ingested by their insect hosts. Presumably, this effect is mediated via the insect host rather than a direct effect of the plant, although a direct effect cannot *a priori* be excluded.[30]

Table 9.1 Insects showing facultative diapause in response to host-plant variables

Insect species	Stage	Diapause inducing factor	Reference
Lepidoptera			
Chilo spp.	Larva	Low protein and water content	39
Coniesta ignefusalis	Larva	Low protein and water content	45
Danaus plexippus	Adult		11
Coleoptera			
Leptinotarsa decemlinetata	Adult	Aged host plant	9, 14
Pectinophora gossypiella	Larva	Lipid, low moisture	2
Callosobruchus maculatus	Adult	Absence of host-plant flowers	48
Diptera			
Bactrocera oleae	Adult	Absence of olive fruits	20
Heteroptera			
Eurydema rugosa	Adult	Absence of host-plant leaves	27
Hymenoptera			
Aphid parasitoids		Food plant of host	30

Several other instances of diapause induction in plant-feeding insects in response to food quality have been reported in the literature but the nature of the effective cues is in most cases unknown (Table 9.1).[5,41] Changes in moisture content and lipid levels have sometimes been found to contribute to diapause induction,[5] but many other factors, including plant growth regulators, may also act as signals for timing the neuroendocrine processes that regulate diapause.

9.2 Reproduction

Herbivorous insects often oviposit on or near their larval food plants. For various reasons it may be a good strategy to postpone egg production and mating behaviour until the female insect is sure that food plants at the right developmental stage are available. Indeed, in many species maturation of the gonads and mating behaviour are delayed in the absence of stimulating cues from host plants.

9.2.1 Maturation

Figure 9.5 Egg-laying activity in desert locusts (*Schistocerca gregaria*) as a function of age and type of food. All insects were fed on cabbage leaves. Insects were fed old (senescent) leaves or fresh (full-grown normal) leaves. One group of insects was fed old food supplemented with 1 μg gibberellin A_2 (gib) per locust per day. Another group of insects was fed old food, but was treated externally with 1 μl of eugenol on the seventh day after the final moult. (From Ellis *et al.*, 1965.)[10]

In natural populations the reproductive activity of desert locust (*Schistocerca gregaria*) adults can be delayed for periods of up to 9 months. Within a population, however, the onset of reproduction is remarkably well synchronized between individuals. Some environmental cue must trigger the maturation process, with the result that after 1–2 weeks, coinciding with the beginning of the rainy season, all females are ready to oviposit. The signal stimulating egg development comes from the food. When the adult locusts eat bursting buds from some *Commiphora* species, such as *C. myrrha*, compounds that initiate sexual maturation are ingested. *Commiphora* buds have been known from ancient times to contain large amounts of essential oils. The supposition that some oil constituents—terpenoids such as pinene (**46**), eugenol (**22**), and limonene (**34**)—induce sexual development has been verified in laboratory experiments. When young adults are fed senescent cabbage leaves only, sexual maturation is suppressed for extensive periods of time (Fig. 9.5). When normal full-grown foliage is given, egg-laying starts 3 weeks after the last moult. Following a single treatment of the insects with eugenol, or supplementing the food with gibberellic acid (**25**) (a plant growth hormone present in high concentrations in new leaves), normal egg production occurs on the deficient (senescent) food (Fig. 9.5).[10] These compounds apparently serve as signals that in nature activate the neuroendocrine system, thereby stimulating sexual development.

Similar associations, although often less marked, have been found in other insect groups. The prereproductive period in female diamondback moths (*Plutella xylostella*) becomes longer when no host plant (cabbage) is present. Experiments employing host-plant specific volatiles have indicated that the host influence on ovarian development can be simulated by a single host-plant odour constituent, allyl isothiocyanate (Table 9.2).[17]

In the bean weevil *Acanthoscelides obtectus* host odours are not effective and oogenesis is stimulated only after palpal contact with either bean seeds, leaves, or pods. Beans are extensively palpated, causing increased oogenesis within days. When the beans are varnished, however, or when the

Table 9.2 Effect of presence of host plant after 3 days on oogenesis in *Plutella xylostella* females (modified from Hillyer and Thorsteinson, 1969)[17]

Stimulus	No. of females	Mean no. of eggs per ovariole
Control (no plant)	47	2.4
Cabbage plant	38	5.7
Allyl isothiocyanate 0.1 ml	32	4.3
0.2 ml	31	5.1

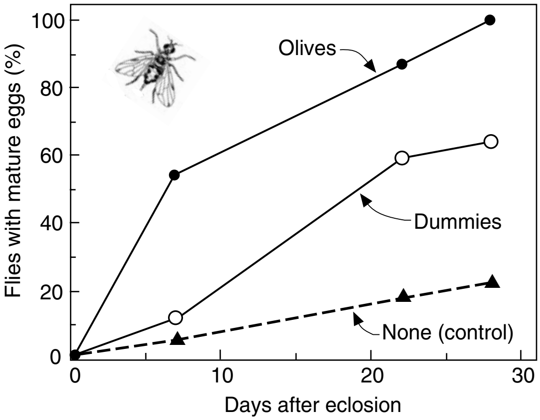

Figure 9.6 Egg maturation of female olive flies (*Dacus oleae*) in the presence or absence of olive fruits or dummies (orange-coloured wax domes). (From Koveos and Tzanakakis, 1990.)[19]

Table 9.3 Insects in which oogenesis is stimulated by kairomones from their host plants

Insect species	Host range	Host plant	Reference
Orthoptera			
Acrididae			
Schistocerca gregaria	G	Various	10
Homoptera			
Aphalaridae			
Euphyllura phillyreae	S	Oleaceae	32
Lepidoptera			
Plutellidae			
Acrolepiopsis assectella	S	Leek	*
Plutella xylostella	S	Brassicaceae	17, 29
Pyralidae			
Homoeosoma electellum	S	Sunflowers	25
Gelechiidae			
Scrobipalpa ocellatella	S	Beet	*
Phtorimaea operculella	S	Potato	*
Sitotroga cerealella	S	Grains	*
Tortricidae			
Zeiraphera diniana	S	Larch	*
Diptera			
Tephritidae			
Philophylla heraclei	S	Apiaceae	*
Dacus oleae	S	Olive fruits	*, 19
Chloropidae			
Oscinella frit	S	Grasses	*
Coleoptera			
Bruchidae			
Acanthoscelides obtectus	S	Bean seeds	*, 31
Careydon serratus	S	Peanut (seeds)	6

G, generalist; S, specialist.

* See Robert, 1986,[38] for references.

maxillary palps are ablated, no egg ripening occurs, indicating that these palps have a crucial function in the perception of gustatory stimuli. Indeed, bean seed washings elicit an oogenesis response in weevils, albeit to a lesser degree than beans themselves.[31] Although chemical cues, olfactory as well as gustatory, generally play a paramount role in stimulating sexual maturation, the effects of physical factors cannot be excluded (Fig. 9.6). In walnut flies (*Rhagoletis juglandis*), for instance, egg development begins earlier and progresses faster in the presence of walnut fruit than in the absence of host-specific cues. Enhancement of oogenesis is also observed when the insects are exposed to yellow spheres, mimicking their natural host. Evidently visual (and possibly tactile) host-fruit stimuli produce physiological changes in *R. juglandis* females that lead to an acceleration of egg development.[21]

In the literature some other instances of accelerated egg maturation due to kairomones from the host plant have been reported (Table 9.3).[38] All but one case involve specialist feeders—a fact that is probably not fortuitous. Host-plant effects, as described above for food specialists but also for the polyphagous desert locust, may be considered as 'anticipation' of the availability of sufficient food for the offspring. Flexibility in timing of reproductive development contributes to a life-history strategy that allows adaptation to unpredictable changes in host availability.

9.2.2 Mating behaviour

Amateur entomologists rearing moths in captivity have known for a long time that mating is often stimulated by the presence of host plants. Riddiford and Williams reported the first experimental proof of the influence of host plants on mating in the polyphemus moth *Antheraea polyphemus*.[37] Copulation in this insect occurred only in the presence of volatiles from oak leaves, their host plant. Since then, several examples have been documented of female moths that begin pheromone release or 'calling' at a younger age, begin calling earlier in the night, and spend more time calling when in the vicinity of their host plants.[24,36,47] In the small ermine moth *Yponomeuta cagnagellus*, a long-lived species that reaches sexual maturity about 1 week after emergence, host-plant volatiles act as a releaser of calling behaviour. In the absence of host material, calling is delayed or even permanently suppressed (Fig. 9.7).[16] In this case the association between the odour of a suitable host and the age at which females initiate calling may contribute to the speciation process within the genus *Yponomeuta*, as it promotes reproductive isolation among sibling species that use different plant species as larval food.[26]

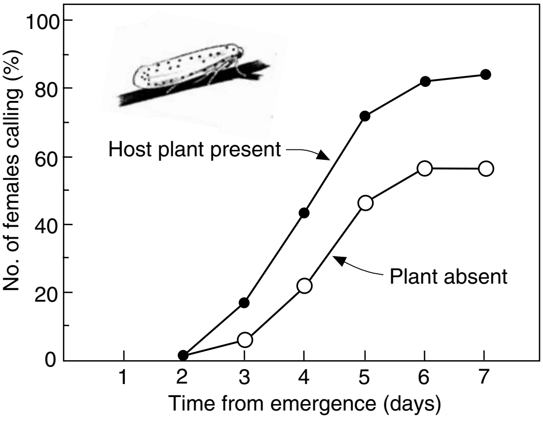

Figure 9.7 Calling behaviour in females of *Yponomeuta evonymellus* in the presence or absence of host plants. (From Hendrikse and Vos-Bünnemeyer, 1987.)[16]

Plant odours also affect male insects. In several nocturnal species males respond more strongly to female sex attractants when they occur mixed with green-leaf volatiles.[22] Green-leaf volatiles and some terpenoids can interact with pheromones at the receptor level, thereby modulating the sensitivity of the pheromone receptor.[28,42] Field experiments likewise have shown enhanced responsiveness to sex pheromones when combined with host-plant volatiles. Male moths of beet armyworms (*Spodoptera exigua*), for instance, were more attracted to traps baited with combinations of the female sex pheromone and volatile plant compounds than to those with pheromone alone, suggesting that some host-plant volatiles enhance their orientation response to females calling from their host plants.[7]

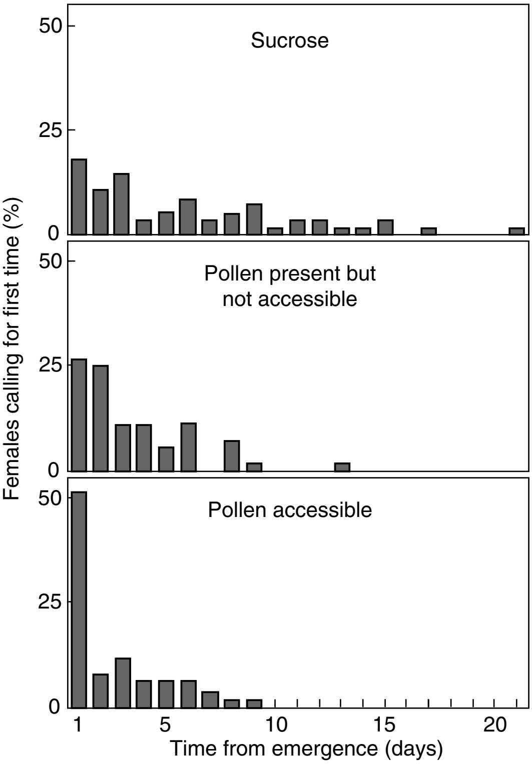

Figure 9.8 Age at which females of sunflower moths (*Homoeosoma electellum*) initiate calling behaviour for the first time. Insects were held in the presence of sucrose alone, or sucrose and sunflower pollen that was not directly accessible, or sucrose and accessible pollen. (From McNeil and Delisle, 1989b.)[25]

Sometimes a very specific part of the host plant may accelerate female calling behaviour, as in the sunflower moth *Homoeosoma electellum*, which oviposits preferentially in newly opened flowers. Most females initiate calling for the first time during the first day after emergence, whereas in the absence of pollen calling behaviour may be delayed for periods of up to 2 weeks (Fig. 9.8). Neonate larvae of this species require free pollen which, due to biotic and abiotic influences, will be available during a short time-window. The ability rapidly to initiate or delay calling behaviour (and concomitant ovarian development) in response to pollen availability permits the moth to cope with the unpredictability of food for its offspring.[25]

As yet, knowledge of which plant chemicals stimulate calling behaviour (and thus act as kairomones) is scarce. Conversely, new insights have recently been gained into the physiological mechanisms that control the production of pheromone and calling behaviour.[34] Females of the corn earworm *Helicoverpa zea*, like several other species, locate a host plant prior to mating. Once they have found a suitable food source for their offspring, pheromone production and its release is started. Calling behaviour appears to be elicited by volatiles emanating from the silk of corn ears. Some of their purified components, for example phenylacetaldehyde, evoke the same response. Ethylene, a plant hormone widely involved in fruit ripening, is also effective (Fig. 9.9).

This compound might act as a common host cue, because *H. zea* larvae feed on the fruiting parts of many different plants.[36] Treatment of moth antennae with silver nitrate (silver ions are known to inhibit ethylene responses in plants) suppressed pheromone release in the presence of ethylene, suggesting the involvement of ethylene receptors in the female olfactory system.

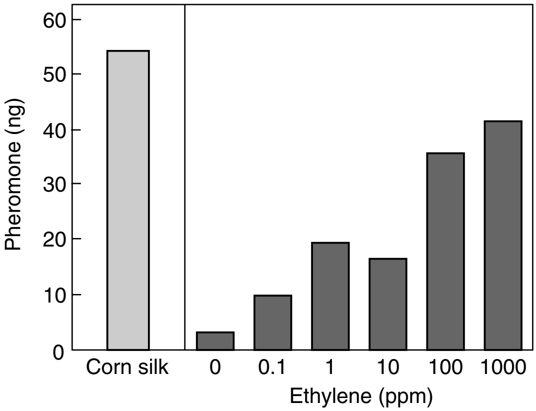

Figure 9.9 Pheromone (*Z*-11-hexadecenal) production (ng per female per 18 h) in *Helicoverpa zea* in the presence of (*left*) corn silk or (*right*) various concentrations of ethylene. (Data from Raina *et al*., 1992.)[36]

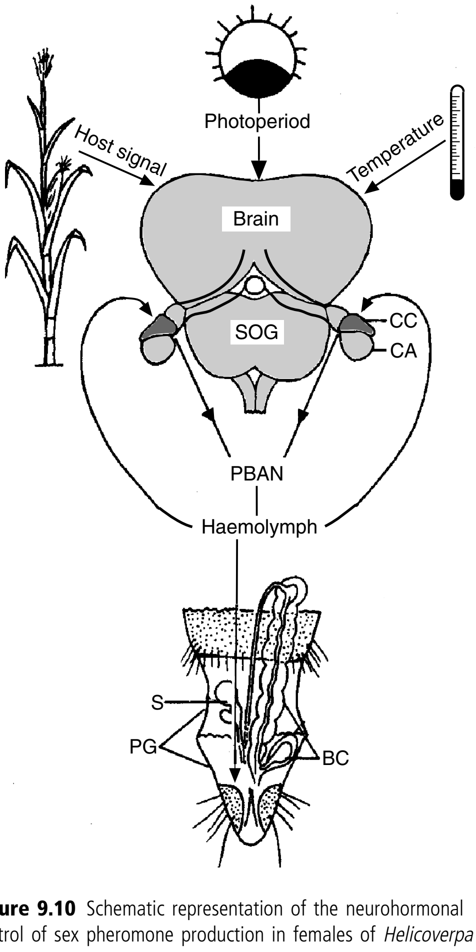

Figure 9.10 Schematic representation of the neurohormonal control of sex pheromone production in females of *Helicoverpa zea*. Pheromone biosynthesis activating neuropeptide (PBAN) is produced in the suboesophageal ganglion (SOG) and transferred to the corpora cardiaca (CC). External factors, such as host-plant odour, photoperiod, and temperature control its release from the corpora cardiaca into the haemolymph, and it then stimulates pheromone production in the abdominal pheromone gland (PG). BC, bursa copulatrix; S, spermatheca; CA, corpora allata. (Redrawn from Raina, 1988.)[35]

When stimulated by host-plant odours, corn earworm females release from their corpora cardiaca a pheromone biosynthesis activating neuropeptide (PBAN), which stimulates the production of pheromone in the abdominal glands (Fig. 9.10).[33,36] PBAN acts directly on pheromone gland cells via calcium and cyclic adenosine monophosphate (cAMP) as second messengers. The latter compound stimulates the biochemical machinery that produces the pheromone.[3]

Not all insects regulate pheromone biosynthesis by using PBAN. Barkbeetles, for example, increase pheromone biosynthesis after feeding on a host tree via juvenile hormone, which is released form the corpora allata.[46]

The examples given suffice to show that in a number of lepidopteran species virgin females first locate a suitable host before they start to attract males. Mating initiates egg production. In this way short-lived insects optimally exploit their limited time and energy resources.

9.3 Conclusions

During the past 50 years the role of plant chemicals in host-plant selection by ovipositing female insects and subsequently in food recognition by their hungry larvae has been documented extensively (see Chapters 6 and 7). The role of host plants in timing insect development has in comparison received little attention. In this chapter some examples of insects that have adapted their life cycle to that of their host plants have been discussed briefly. Although only a few examples of this type of plant effect on insects are known, from a taxonomic point of view they cover a strikingly broad variety of insects, indicating that this may be a fairly common phenomenon (see, for instance, Table 9.3). Not only insects belonging to different orders but also those in different developmental stages appear to be affected, a further indication of the importance of the capacity to tune in to subtle host signals.

In contrast to insect parasitoids or insect parasites living on vertebrate hosts, herbivorous insects must use signals from host organisms with regulatory systems that are totally different from those regulating their own growth and development. Whereas insect parasitoids can employ host hormones identical to their own internal signal molecules as cues to synchronize their life cycles, and rabbit fleas use host hormones to adapt their reproductive cycles to that of their hosts, herbivores must rely on signals that are in no way related to physiologically familiar compounds. The signals from a plant advertizing its presence or details about its physiological state are predominantly chemicals. Sometimes they are host-plant specific; in other cases they are more general compounds such as plant hormones.

The identity of most of the relevant chemicals largely remains to be elucidated, and the way in which insects detect and decipher the chemical signals also needs further investigation. The chain of events taking place inside the insect, likewise, leaves many open questions, but at least there is compelling evidence that all physiological and behavioural responses are mediated by the neuroendocrine system.

A striking aspect of the described effects of plant signals is that, unlike responses to photoperiod, they rarely evoke an all-or-nothing reaction. In most cases development is only accelerated, and at least part of the population will complete development, albeit with some delay, even in the continued absence of the plant signal.

We foresee that the topic of fine adjustments of insect life cycles to host-plant phenology will give an extra dimension—one of great richness—to the field of insect–plant relationships.

9.4 References

1. Akino, T., Nakamura, K.I., and Wakamura, S. (2004). Diet-induced chemical phytomimesis by twig-like caterpillars of *Biston robustum* Butler (Lepidoptera: Geometridae). *Chemoecology*, **14**, 165–74.
2. Bull, D.L. and Adkisson, P.L. (1960). Certain factors influencing diapause in the pink bollworm *Pectinophora gossypiella. Journal of Economic Entomology*, **53**, 793–8.
3. Choi, M.Y. and Jurenka, R.A. (2004). PBAN stimulation of pheromone biosynthesis by inducing calcium influx in pheromone glands of *Helicoverpa zea. Journal of Insect Physiology*, **50**, 555–60.
4. Danilevski, A.S. (1965). *Photoperiodism and seasonal development of insects*. Oliver & Boyd, London.

5. Danks, H.V. (1987). *Insect dormancy: an ecological perspective*. Biological Survey of Canada, Ottawa.
6. Delobel, A. (1989). Influence des gousses d'arachide (*Arachis hypogea*) et de l'alimentation imaginale sur l'ovogénèse, l'accouplement et la ponte chez la bruche *Careydon serratus*. *Entomologia Experimentalis et Applicata*, **52**, 281–9.
7. Deng, J.-Y., Wei, H.-Y., Huang,Y.-P., and Du, J.-W. (2004). Enhancement of attraction to sex pheromones of *Spodoptera exigua* by volatile compounds produced by host plants. *Journal of Chemical Ecology*, **30**, 2037–45.
8. Dettner, K. (1989). Chemische Ökologie. Ein interdisziplinäres Forschungsgebiet zwischen Biologie und Chemie. *Zeitschrift für Umweltchemie und Ökotoxikologie*, **4**, 29–36.
9. De Wilde, J., Bongers, W., and Schooneveld, H. (1969). Effects of hostplant age on phytophagous insects. *Entomologia Experimentalis et Applicata*, **12**, 714–20.
10. Ellis, P.E., Carlisle, D.B., and Osborne, D.J. (1965). Desert locusts: sexual maturation delayed by feeding on senescent vegetation. *Science*, **149**, 546–7.
11. Goehring, L. and Oberhauser, K.S. (2002). Effects of photoperiod, temperature, and host plant age on induction of reproductive diapause and development time in *Danaus plexippus*. *Ecological Entomology*, **27**, 674–85.
12. Greene, E. (1989). A diet-induced developmental polymorphism in a caterpillar. *Science*, **243**, 643–6.
13. Hardie, J. and Lees, A.D. (1985). Endocrine control of polymorphism and polyphenism. In *Comprehensive insect physiology, biochemistry and pharmacology*, Vol. 8 (ed. G.A. Kerkut and L.I. Gilbert), pp. 441–490. Pergamon Press, Oxford.
14. Hare, J.D. (1983). Seasonal variation in plant–insect associations: utilization of *Solanum dulcamara* by *Leptinotarsa decemlineata*. *Ecology*, **64**, 345–61.
15. Harrewijn, P. (1978). The role of plant substances in polymorphism of the aphid *Myzus persicae*. *Entomologia Experimentalis et Applicata*, **24**, 198–214.
16. Hendrikse, A. and Vos-Bünnemeyer, E. (1987). Role of the host-plant stimuli in sexual behaviour of the small ermine moths *(Yponomeuta)*. *Ecological Entomology*, **12**, 363–71.
17. Hillyer, R.J. and Thorsteinson, A.J. (1969). The influence of the host plant or males on ovarian development or oviposition in the diamondback moth *Plutella maculipennis* (Curt.). *Canadan Journal of Zoology*, **47**, 805–16.
18. Kawada, K. (1987). Polymorphism and morph determination. In *Aphids, their biology, natural enemies, and control; world crop pests*, Vol. 2A (ed. A.K. Minks and P. Harrewijn), pp. 255–268. Elsevier, Amsterdam.
19. Koveos, D.S. and Tzanakakis, M.E. (1990). Effect of the presence of olive fruit on ovarian maturation in the olive fruit fly, *Dacus oleae*, under laboratory conditions. *Entomologia Experimentalis et Applicata*, **55**, 161–8.
20. Koveos, D.S. and Tzanakakis, M.E. (1993). Diapause aversion in the adult olive fruit-fly through effects of the host fruit, bacteria, and adult diet. *Annals of the Entomological Society of America*, **85**, 668–73.
21. Lachmann, A.D. and Papaj, D.R. (2001). Effect of host stimuli on ovariole development in the walnut fly, *Rhagoletis juglandis* (Diptera, Tephritidae). *Physiological Entomology*, **26**, 38–48.
22. Landolt, P.J. and Phillips, T.W. (1997). Host plant influences on sex pheromone behavior of phytophagous insects. *Annual Review of Entomology*, **42**, 371–91.
23. Lees, A.D. (1961). Clonal polymorphism in aphids. *Symposia of the Royal Entomological Society of London*, **1**, 68–79.
24. McNeil, J.N. and Delisle, J. (1989a). Are host plants important in pheromone-mediated mating systems of Lepidoptera? *Experientia*, **45**, 236–40.
25. McNeil, J.N. and Delisle, J. (1989b). Host plant pollen influences calling behavior and ovarian development of the sunflower moth, *Homoeosoma electellum*. *Oecologia*, **80**, 201–5.
26. Menken, S.B.J., Herrebout, W.M., and Wiebes, J.T. (1992). Small ermine moths (*Yponomeuta*): their host relations and evolution. *Annual Review of Entomology*, **37**, 41–66.
27. Numata, H. and Yamamoto, K. (1990). Feeding on seeds induces diapause in the cabbage bug, *Eurydema rugosa*. *Entomologia Experimentalis et Applicata*, **57**, 281–4.
28. Ochieng, S.A., Park, K.C., and Baker, T.C. (2002). Host plant volatiles synergize responses of sex pheromone-specific olfactory receptor neurons in male *Helicoverpa zea*. *Journal of Comparative Physiology A*, **188**, 325–33.
29. Pittendrigh, B.R. and Pivnick, K.A. (1993). Effects of a host plant, *Brassica juncea*, on calling behaviour and egg maturation in the diamondback moth, *Plutella xylostella*. *Entomologia Experimentalis et Applicata*, **68**, 117–26.
30. Polgár, L.A. and Hardie, J. (2000). Diapause induction in aphid parasitoids. *Entomologia Experimentalis et Applicata*, **97**, 21–7.
31. Pouzat, J. (1978). Host plant chemosensory influence on oogenesis in the bean weevil, *Acanthoscelides obtectus* (Coleoptera: Bruchidae). *Entomologia Experimentalis et Applicata*, **24**, 601–8.

32. Prophetou-Athanasiadou, D.A. (1993). Diapause termination and phenology of the olive psyllid, *Euphyllura phillyreae* on two host plants in coastal northern Greece. *Entomologia Experimentalis et Applicata*, **67**, 193–7.
33. Rafaeli, A. (2001). Neuroendocrine control of pheromone biosynthesis in moths. *International Review of Cytology*, **213**, 49–92.
34. Rafaeli, A. (2005). Mechanisms involved in the control of pheromone production in female moths: recent developments. *Entomologia Experimentalis et Applicata*, **115**, 7–15.
35. Raina, A.K. (1988). Selected factors influencing neurohormonal regulation of sex pheromone production in *Heliothis* species. *Journal of Chemical Ecology*, **14**, 2063–9.
36. Raina, A.K., Kingan, T.G., and Mattoo, A.K. (1992). Chemical signals from host plant and sexual behavior in a moth. *Science*, **255**, 592–4.
37. Riddiford, L.M. and Williams, C.M. (1967). Volatile principle from oak leaves: role in sex life of the polyphemus moth. *Science*, **155**, 589–90.
38. Robert, P.C. (1986). Les relations plantes–insectes phytophages chez les femelles pondeuses: le rôle des stimulus chimiques et physiques. Une mise au point bibliographique. *Agronomie*, **6**, 127–42.
39. Scheltes, P. (1978). The condition of the host plant during aestivation–diapause of the stalk borers *Chilo partellus* and *Chilo orichalcociliella* in Kenya. *Entomologia Experimentalis et Applicata*, **24**, 479–88.
40. Sethi, S.L. and Swenson, K.G. (1967). Formation of sexuparae in the aphid *Eriosoma pyricola*, on pear roots. *Entomologia Experimentalis et Applicata*, **10**, 97–102.
41. Steinberg, S., Podoler, H., and Applebaum, S.W. (1992). Diapause induction in the codling moth, *Cydia pomonella*: effect of larval diet. *Entomologia Experimentalis et Applicata*, **62**, 269–75.
42. Stelinski, L.L., Miller, J.R., Ressa, N.E., and Gut, L.J. (2003). Increased EAG responses of tortricid moths after prolonged exposure to plant volatiles: evidence for octopamine-mediated sensitization. *Journal of Insect Physiology*, **49**, 845–56, 1083.
43. Sutherland, O.R.W. (1967). Role of host plant in production of winged forms by a green strain of pea aphid *Acyrthosiphon pisum* Harris. *Nature*, **216**, 387–8.
44. Sutherland, O.R.W. and Mittler, T.E. (1971). Influence of diet composition and crowding on wing production by the aphid *Myzus persicae Journal of Insect Physiology*, **17**, 321–8.
45. Tanzubil, P.B., Mensah, G.W.K., and McCaffery, A.R. (2000). Diapause initiation and incidence in the millet stem borer, *Coniesta ignefusalis* (Lepidoptera: Pyralidae): the role of the host plant. *Bulletin of Entomological Research*, **90**, 365–71.
46. Tillman, J.A., Seybold, S.J., Jurenka, R.A., and Blomquist, G.J. (1999). Insect pheromones—an overview of biosynthesis and endocrine regulation. *Insect Biochemistry and Molecular Biology*, **29**, 481–514.
47. Yan, F., Bengtsson, M., and Witzgall, P. (1999). Behavioral response of female codling moths, *Cydia pomonella*, to apple volatiles. *Journal of Chemical Ecology*, **25**, 1343–50.
48. Zannou, E.T., Glitho, I.A., Huignard, J., and Monge, J.P. (2003). Life history of flight morph females of *Callosobruchus maculatus* F.: evidence of a reproductive diapause. *Journal of Insect Physiology*, **49**, 575–82.

CHAPTER 10

Ecology: living apart together

Central themes in ecology are how organisms interact with the biotic and abiotic environment and how these interactions can explain the composition and dynamics of communities.[117,141] These aspects can be investigated at different levels of biological organization, but an important challenge for ecologists is to link these. For instance, community ecologists analyse how species composition of communities fluctuates in the context of the underlying population dynamics. In addition, behavioural ecologists investigate how individual insects respond to their biotic and abiotic environment, and whether these responses can be understood in the context of costs and benefits in terms of fitness or offspring production. An individual's characteristics, which can affect their interactions with other organisms and thus population dynamics and community composition, are determined by processes at the individual, cellular, and subcellular level. How gene expression affects an individual's phenotype and subsequently interactions among individuals, within populations, and finally community processes is the topic of the new research field of ecological or environmental genomics and will be a major topic in biology in the twenty-first century.

In Chapter 2 we saw that each plant serves as food for a range of insect herbivore species. In addition, carnivorous arthropods may inhabit plants or utilize plant products. Moreover, insect

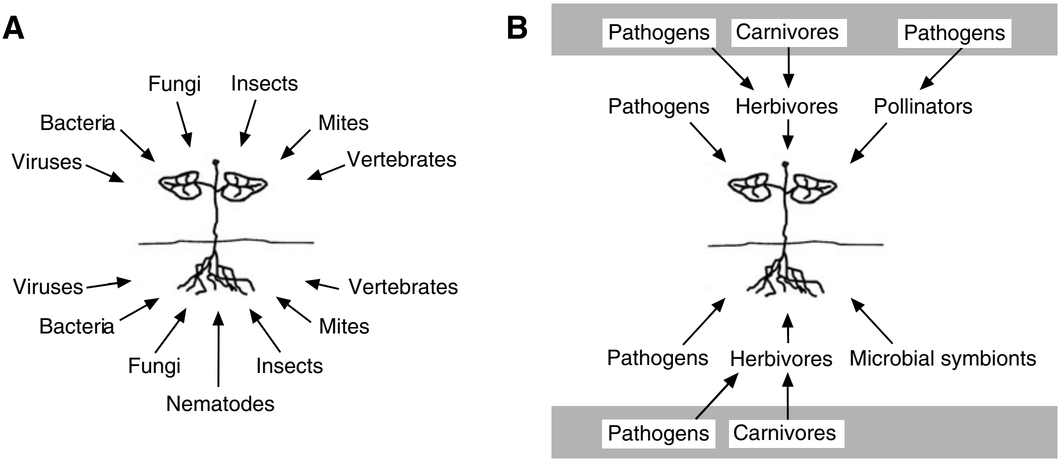

Figure 10.1 Plants live in complex communities. (**A**) They are attacked by a wide range of organisms from very different taxonomic groups. (**B**) Each of these attackers has its own natural enemies and there are connections among the attackers as well. In addition, there are interactions with mutualists and symbionts such as pollinators and mycorrhizae.

communities on plants are faced with a multitude of other plant attackers, ranging from viruses and organisms, such as bacteria, fungi, nematodes, molluscs, and mites, to vertebrates, such as birds and mammals (Fig. 10.1). Again, each of these is associated with antagonists. Finally, plants interact with competing plants for resources and with symbionts such as mycorrhizae or root-nodule bacteria. Thus, insect–plant communities are embedded in a complex setting.

Interactions of plants with insects take place above and below ground, but in neither of these environments do plants interact with insects exclusively. In this chapter we explore how plants and insects interact among themselves and how other organisms can influence these interactions. Finally we discuss how multitrophic interactions (interactions involving several trophic levels) between plants and insects are embedded in food webs and communities, and how insect–plant interactions affect community dynamics and are affected by community dynamics.

10.1 Effects of plants on insects

In previous chapters we saw that plants affect herbivorous insects in various ways. Plants are not only food, but they also influence herbivorous insects through, for example, physical structures, their three-dimensional architecture, and chemical information. Moreover, plants may interact in similar ways with pollinators and carnivorous insects, and this is affected by and affects interactions with their herbivores.[36,148] The degree to which carnivores inhabit plants can be influenced by plant characteristics. In other words, plants may provide an enemy-free or enemy-dense space to herbivores. Consequently, plant defences can be characterized as direct or indirect. Direct defence is mediated by plant characteristics that affect the herbivore's biology, such as toxins or thorns. In contrast, indirect defence improves the performance of carnivorous insects, for example through the provision of shelter, alternative food, or infochemicals (Fig. 10.2).[35] Plant characteristics that turn a plant into enemy-dense space for herbivores are a component of indirect plant defence.

Pollinators visit plants during flowering periods and plants stimulate this through various characteristics such as visual cues, odours, and food rewards (see Chapter 12). Moreover, flowers may be an enemy-dense or enemy-free space for pollinators, and this may influence the success of pollination.[43]

Each interaction may modify other interactions within the community and, therefore, one cannot consider individual interactions exclusively and simply add them together to get an impression of

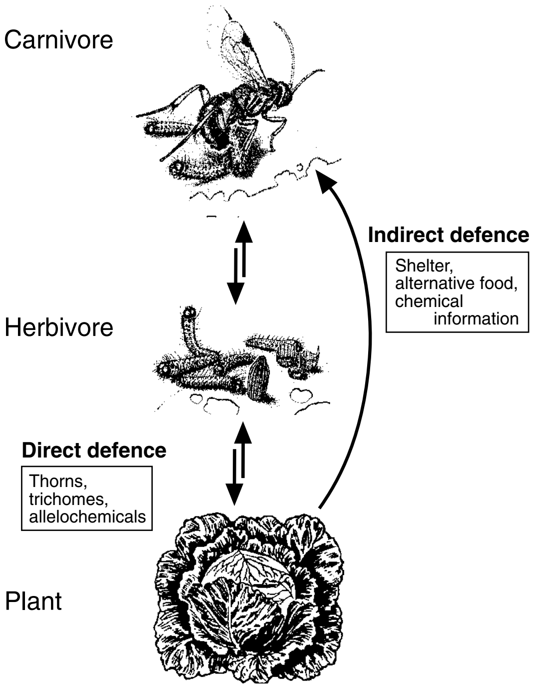

Figure 10.2 Plants can defend themselves against herbivores by direct defence that affects the herbivore or by indirect defence that enhances the effectiveness of carnivorous enemies of the herbivore.

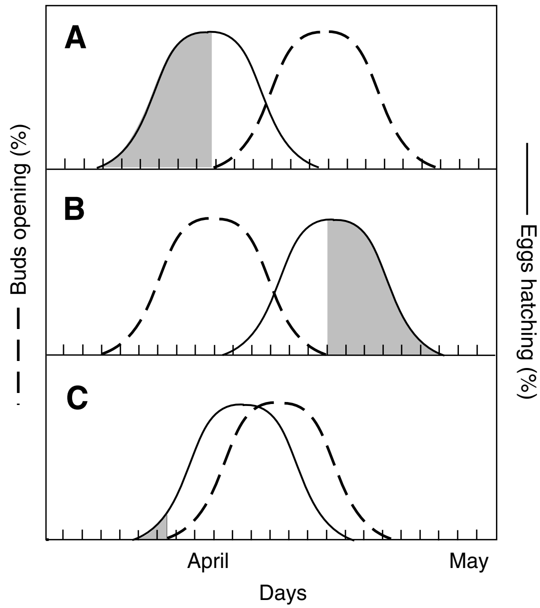

Figure 10.3 Timing of *Operophthera brumata* egg hatch and oak bud break. Shaded area indicates starvation of larvae. (From Feeny, 1976.)[46]

community organization. In this section we address the effects of individual plant characteristics on carnivores and pollinators. The effects of plants on carnivores may be mediated directly by plant characteristics, such as the influence of plant trichomes on carnivore movement (see Chapter 3). In addition, plants may affect carnivores indirectly through their effects on herbivores. For instance, plant quality may affect herbivore size and this may influence oviposition choices by parasitoids. Plant effects on host size may therefore affect interactions of the herbivore with its parasitoids and thus have important consequences for the parasitoid community.[93] Both types of effect are covered in the following sections.

10.1.1 Plant phenology

Phenology of the host plant may be crucial for the performance of herbivorous insects. Climate changes disrupting the synchronization between insect and plant phenology may strongly influence insect population dynamics. For example, most foliage-feeding lepidopterans, such as the larvae of the winter moth *Operophthera brumata*, are able to attack oaks (*Quercus robur*) only from just after bud break until the extension of the first set of leaves. Figure 10.3 shows that if bud opening precedes egg hatch (B) or egg hatch precedes bud opening (A), a large proportion of the larvae starve. Thus, the more precisely the timing of the two events coincides (C), the better the performance of the larvae (Fig. 10.4) and the stronger the defoliation.[45,46] Clearly, individual trees that bud either very early or very late may remain unattacked by these insects through 'phenological escape'. The importance of host-plant phenology in this system in the light of climate change has recently been demonstrated. Spring temperatures have increased over the past 25 years without a decrease in the incidence of freezing spells in winter, and different species may respond in dissimilar ways. The climatic change has resulted in an increasingly poor synchrony between winter moth egg-hatching and oak bud burst in the Netherlands, most likely as a result of changes in the relationship between

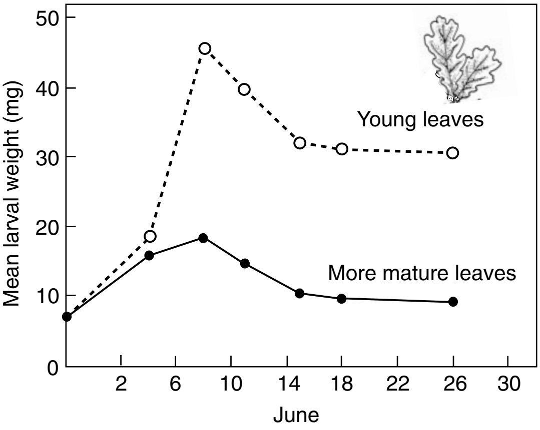

Figure 10.4 Mean fresh weight of fourth-instar *Operophthera brumata* larvae reared on young and more mature oak leaves. The initial rise in weight representing fourth- and fifth-instar larval feeding phase is followed by a decline during the prepupal phase until pupation is complete. The difference in performance is due to the decrease in protein content and increase in tannin content of oak leaves with age. (From Feeny, 1976.)[46]

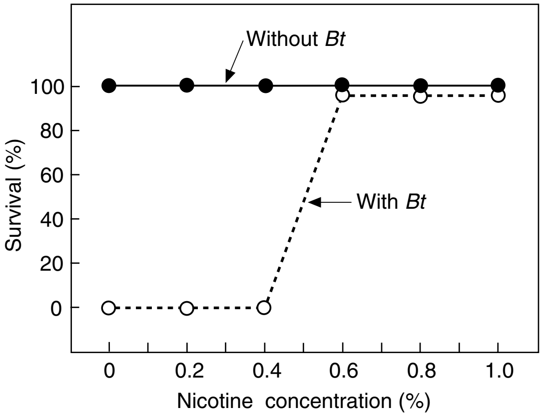

Figure 10.5 Survival to pupation of *Manduca sexta* larvae reared on six concentrations of nicotine incorporated into a synthetic diet with or without *Bacillus thuringiensis*. (Modified from Krischik *et al*., 1988.)[85]

abiotic variables that influence oak and moth phenology.[179]

10.1.2 Plant chemistry

The effects of secondary plant metabolites on herbivores were addressed extensively in Chapter 5. It is clear that these chemicals can retard the development of herbivorous insects, can intoxicate and kill them, or can be sequestered by herbivorous insects. Which of these effects occurs depends on the specific metabolite–herbivore combination. Each of these effects can affect interactions between herbivores and their natural enemies. When secondary metabolites retard a herbivore's development, the insect may remain longer in a stage that is susceptible to attack by carnivores. For example, the leaf beetle *Galerucella* experiences a higher mortality rate from natural enemies on willows of suboptimal quality, on which the beetles develop at a lower rate.[57] When two co-occurring insects share a common parasitoid, the one with the slower developmental rate may be locally exterminated as a result of a higher mortality rate inflicted by the parasitoid.[19]

Toxic plant compounds may be exploited by specialist herbivores for their own defence. Specialist herbivores usually tolerate higher levels of toxins and often sequester the defensive plant chemicals. As a result they are better protected against their natural enemies such as pathogens, predators, and parasitoids. This is exemplified by the effects of nicotine in interactions between tobacco, the specialist herbivore *Manduca sexta* (tobacco hornworm), the generalist herbivore *Trichoplusia ni* (cabbage looper), and a pathogenic bacterium, *Bacillus thuringiensis*. The generalist herbivore is adversely affected by nicotine in the plant, whereas nicotine has only minor effects on the fitness of the specialist herbivore. Higher nicotine concentrations in the plant even appear to be beneficial to the specialist herbivore when exposed to the pathogen *B. thuringiensis*. Caterpillars feeding on a high-nicotine diet suffer little mortality from *B. thuringiensis*, in contrast to caterpillars exposed to a diet low in nicotine (Fig. 10.5). Sequestered nicotine may similarly protect specialist herbivores against parasitoids or predators. Among predators and parasitoids, the specialists are better adapted to such defences of their prey/host. As a consequence, specialist herbivores are better protected against generalist natural enemies than against specialist natural enemies.[7]

Herbivory can induce major changes in plant chemistry that involve, for example, toxins and digestibility reducers (see Chapter 4). For instance, mechanical damage or herbivore damage of tobacco plants results in dramatic increases in nicotine concentration, which defends the plants against non-adapted herbivores.[5] However, as we have seen above, specialist herbivores may exploit the plant's chemical defence against their own natural enemies through sequestration. It is interesting to note that plants may reduce the 'misuse' of its defence chemical. When wild tobacco is attacked by the specialist herbivore *M. sexta*, it shows an attenuated induction of nicotine. Elicitors in the regurgitant of this herbivore mediate this attenuated plant response.[78] Thus, it appears that plants may tailor induced changes in secondary metabolites to the type of herbivore that attacks them.

Herbivory may also induce secondary metabolites that benefit the effectiveness of carnivorous enemies of herbivores and thus indirectly benefit the plant. For instance, herbivory induces large quantitative and qualitative changes in the emission of volatiles that attract carnivorous enemies of the herbivores, such as parasitoids and predators.[35,163] Even oviposition by herbivores can induce the emission of carnivore-attracting plant volatiles.[70] To the carnivores, the volatiles provide a solution to an important foraging problem they face: the reliability–detectability problem (Fig. 10.6).[175] Chemicals from their herbivorous victim would obviously be the most reliable cues for carnivores to locate a herbivore. However, herbivores are small components of the environment and are under selection pressure to minimize the emission of cues that can be exploited by their enemies. In contrast, cues from the herbivore's food plant are more abundant because of the larger plant biomass, although their reliability in indicating herbivore presence is low, except for herbivore-induced plant volatiles. Herbivore-induced plant volatiles are not only specific for the plant species, but may be specific for the inducing herbivore species and instar as well. Therefore, herbivore-induced plant volatiles are highly detectable cues that are often reliable in revealing the identity and density of feeding herbivores.[175] The other side of the coin is the observation that herbivore-induced plant volatiles may attract other herbivores, owing to the fact that the damaged plants are much more apparent than

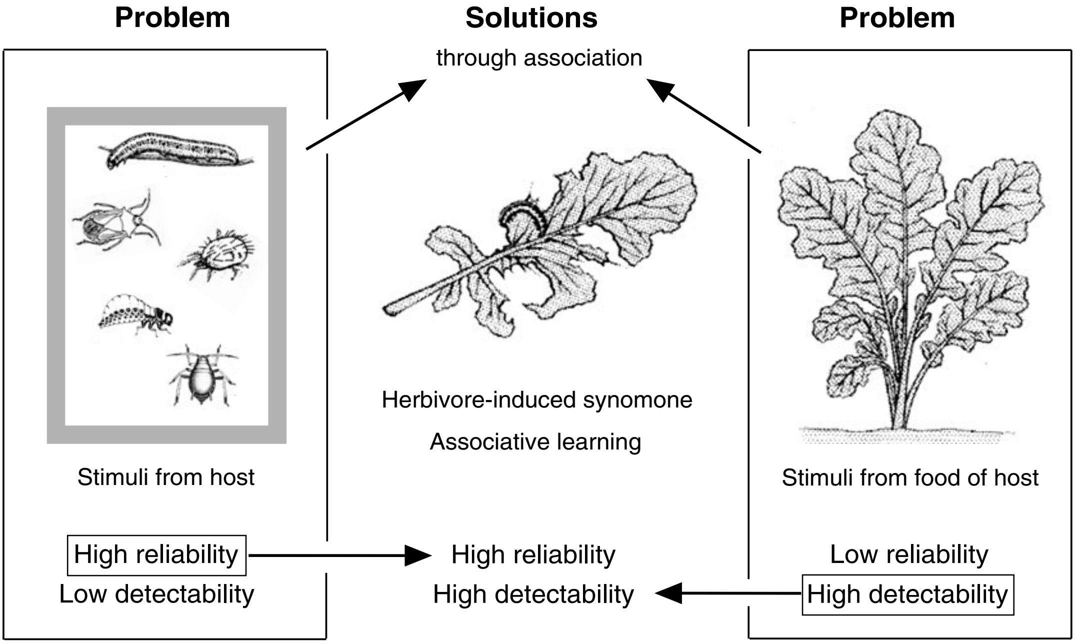

Figure 10.6 Foraging carnivorous arthropods are faced with a reliability–detectability problem related to constitutive chemical information available from organisms at the first and second trophic level. Association is a key solution to this problem. (From Vet *et al.*, 1991.)[174]

undamaged plants, which have a much lower volatile emission rate. The cabbage looper moth *Trichoplusia ni*, for example, is attracted by volatiles from cabbage plants that are infested by conspecific caterpillars, but once the moth has arrived at the odour source she oviposits on nearby uninfested cabbage plants.[87]

Induced changes in plant chemistry can have extensive effects on the composition of the insect fauna community.[153,154]

10.1.3 Plant morphology

Plant morphology can influence the presence and activity of herbivorous as well as carnivorous insects (see Chapter 3). As a consequence, plant morphology affects the relative contribution of herbivorous and carnivorous insects to the community inhabiting the plants. This can have significant effects on herbivore–carnivore interactions and population dynamics. For instance, a simple mutation that results in an architectural change of pea plants has important effects on population dynamics. On the wild-type pea plant, aphids escape predation from coccinellid predators better than on the leafless mutant, because the ladybirds fall off the wild-type variety nearly twice as frequently as they do from the mutant. As a result, the population increase of aphids is much higher on the wild-type than on the mutant plant, under both laboratory and field conditions.[80]

10.1.4 Alternative food

To attract pollinators, plants endow their flowers with nectar. However, nectar and pollen can be consumed by herbivores and carnivores as well. Carnivorous insects such as ants are regular visitors of flowers where they collect nectar (Fig. 10.7A). In an elegant study, Yano demonstrated that flowering plants of the crucifer *Rorippa indica* receive many and long-lasting ant visits, in contrast to control plants from which the flowers have been removed.[190] These ants patrol the plant and when they encounter a herbivorous insect it is killed and taken to the nest. The herbivorous insect that causes most damage to *R. indica* is the small cabbage white *Pieris rapae*, and ant visitation results in a significant reduction of *Pieris* damage (Fig. 10.7B). Ant visits to flowers did not have a cost in terms of reduced pollination: plants with and without ants had a similar seed production. Thus, floral nectar production can indirectly protect plants against herbivorous insects.

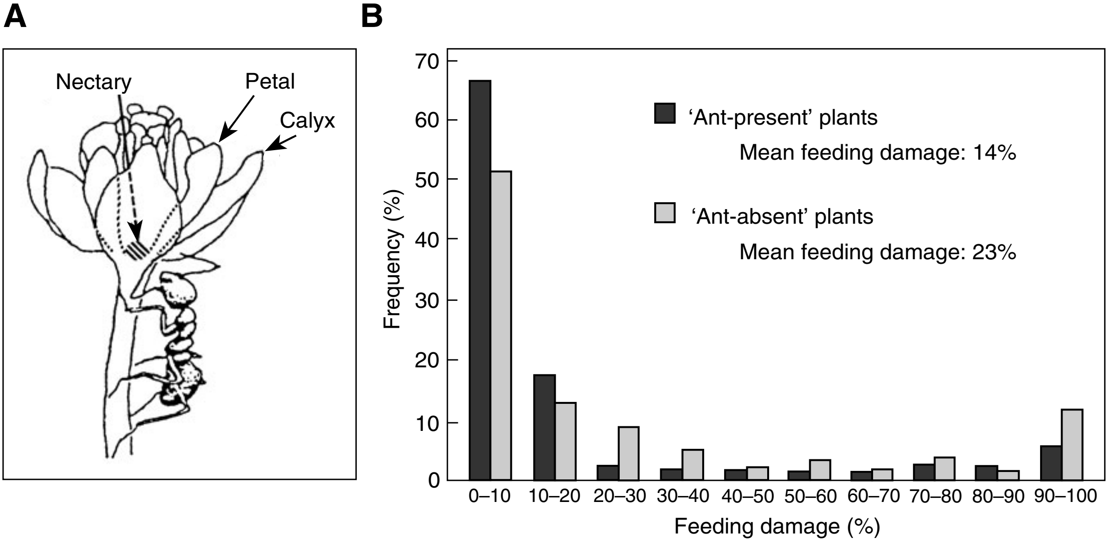

Figure 10.7 (**A**) The ant *Lasius niger* collects floral nectar from a flower of the crucifer *Rorippa indica*. (**B**) The presence of *Lasius niger* ants results in reduced herbivory on *R. indica*; most of the herbivory is done by *Pieris rapae* caterpillars. (From Yano, 1994.)[190]

Pollen can be used as food by several groups of predatory arthropods, such as lacewings, coccinellid beetles, heteropteran bugs, and phytoseiid mites. These carnivores may collect the pollen in the flowers, but in addition they can feed on pollen on the leaves below the flowers on to which the pollen has fallen. In addition, pollen can be a food source for pollinators such as bees and bumble bees, or for herbivores such as lycaenid butterflies, curculionid or chrysomelid beetles, and many thrips species. Thus, pollen mediates a range of interactions and one wonders what the net outcome of these interactions is to an individual plant. First, evidence has become available for a plant–herbivore–carnivore system consisting of male sterile cucumber, western flower thrips (*Frankliniella occidentalis*), and the predatory mite *Iphiseius degenerans*.[172] Both the thrips and its predator can feed and reproduce on pollen as food. In a greenhouse experiment, the addition of cattail pollen to one mature leaf per plant resulted in an increase in the predator population and a decrease in the thrips population. In contrast to the herbivores, the predators were found to aggregate on the pollen-endowed leaves. Thus, although plant pollen is obviously produced for reproductive purposes, it may have an important additional role in indirect defence against herbivores. However, the other side of the coin is that if generalist carnivorous arthropods visit flowers too frequently the flowers become an enemy-dense space and pollinators may learn to avoid them. For instance, honeybees learn to avoid flowers that harbour a dead conspecific or a spider.[43]

Plants can provide carnivores with extrafloral nectar, which is produced from nectaries on leaves or petioles, for example (Fig. 10.8).[83] Its production

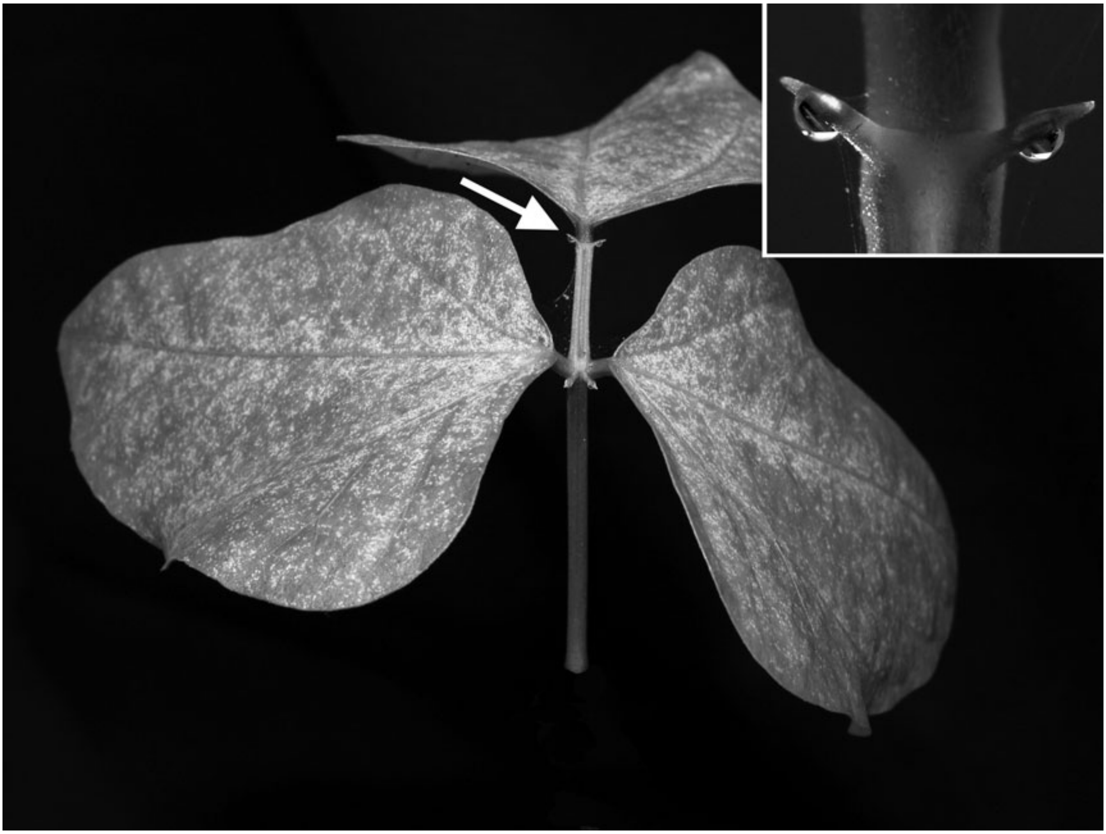

Figure 10.8 Extrafloral nectar secretions from extrafloral nectaries of Lima bean plant that is infested with spider mites (*Tetranychus urticae*). (Photo by H. M. Smid and M. Dicke.)

can be induced by herbivory or mechanical wounding. For instance, the ant-associated plant *Macaranga tanarius*[66] and wild Lima bean (*Phaseolus lunatus*) plants[67] produce extrafloral nectar in response to wounding or jasmonic acid treatment. In contrast to many other induced plant responses (see Chapter 4), induced production of extrafloral nectar seems to be more a local than a systemic phenomenon. For instance, in cotton, the induction of extrafloral nectar takes place mainly at the level of damage, with a slight systemic effect in younger leaves.[184] Carnivorous arthropods such as ants, parasitoids, predatory mites, and spiders are well known consumers of extrafloral nectar,[10,83] and through predation of herbivores these bodyguards can contribute to plant fitness.[131]

10.2 Effects of herbivores on plants

It is obvious that herbivores affect plants, because they consume plant tissues. Insect herbivory can result in plant death in the seedling stage, but mature plants are seldomly killed. However, insect herbivory can have a range of effects on plant characteristics without killing them.[27] The modification of plant characteristics results in altered interactions with herbivorous, and carnivorous insects that affect the population dynamics of plants, herbivores, and carnivores. Feeding may result in altered shoot growth, root growth, flowering, and seed production, and herbivory alters many plant characteristics, such as chemistry, morphology, or the production of extrafloral nectar (see Section 10.1.4). An extreme form of morphological alteration is the induction of galls by some hymenopteran or coleopteran insects (see Chapter 3). Herbivores that eliminate the apical meristem modify plant morphology as a result. For instance, herbivore damage to the apical meristem of the pitcher's thistle *Cirsium pitcheri* results in compensatory growth leading to multi-rosetted plants.[142] Plants have an enormous potential for compensation of herbivory by regrowth.[166] Usually the capability for regrowth is greater when the damage occurs earlier during the season.[160]

Various insect herbivores affect plant reproduction. Seed predators or flower-feeding insects, such as many species of Diptera, Lepidoptera, Coleoptera, and Hymenoptera, have an obvious effect. Moreover, leaf-, root-, and stem-feeding insects can reduce seed production, with most pronounced effects by phloem- or xylem-sucking insects.[27] Seed production may be affected by reducing the resources available for flower and seed production, or by affecting floral and vegetative plant characters, which in turn affect visitation rates by pollinators. For instance, spittlebug feeding on *Rudbeckia hirta* plants reduces flower production as well as pollinator visitation.[61] Pollinators that collect pollen or floral nectar transfer pollen among flowers, and seed predators may disperse plant seeds. For instance, many tropical plants endow their seeds with oil-rich structures (i.e. ant-bodies or elaiosomes) that stimulate ants to transport the seeds to their nest where the seeds are stripped of the nutrients and abandoned.[21]

The effects of insect herbivores on plant population dynamics and plant distribution have long been considered small,[25] but in the past 10 years more and more evidence has shown that such effects can be larger than usually thought. For instance, the removal of flower- and seed-consuming insects from the thistle *Cirsium canescens* resulted in an increase in seed production, an increase in seedlings around plants protected from inflorescence-feeding insects, and a higher number of flowering plants. Thus, the removal of inflorescence-feeding insects resulted in an increase of lifetime fitness of the thistle.[91] Moreover, insect herbivory may influence a plant's distribution. The crucifer *Cardamine cordifolia* is restricted to shaded habitats within its indigenous region, the central Rocky Mountains. Insect herbivory was much more intense in the sun than in the shade, and this is the major factor determining that the plant's distribution is restricted to shaded habitats.[92] A modelling study has suggested that the conditions under which insect herbivores affect the population dynamics of plants are likely to be more widespread than *a priori* expected.[95] This should invite more empirical studies.

Herbivores can also affect plants indirectly through effects on carnivorous insects. Many aphid species are tended by ants that collect the aphid's honeydew. The ants protect the aphids and in addition patrol the plant and attack herbivores. The presence of aphids can greatly benefit the plant through the bodyguard function of ants. In an exciting study, Vrieling and colleagues[182] showed that infestation of *Senecio jacobaea* by a specialist aphid (*Aphis jacobaeae*) resulted in visitation by aphid-tending ants that protected the plant against the specialist herbivorous caterpillar *Tyria jacobaeae*, which is not affected by the plant's direct defence. In contrast to *T. jacobaea*, the aphid *A. jacobaeae* is negatively affected by *S. jacobaea*'s chemical defence (pyrrolizidine alkaloids; PAs). Thus, the presence of the aphids results in a defence against a specialist herbivore that is adapted to the chemical defence of its host plant. Populations of *S. jacobaea* plants are polymorphic for the amount of PAs, and this polymorphism is dependent on disruptive selection. In years with many *T. jacobaeae* caterpillars, plant genotypes with low amounts of PA achieve the highest fitness, whereas in years with few *T. jacobaeae* plant genotypes with high amounts of PA achieve highest fitness.[182]

10.3 Above-ground and below-ground insect–plant interactions

So far, we have almost exclusively regarded above-ground interactions between plants and insects. This reflects the current state of the emphasis in ecology of insect–plant interactions. Studies on below-ground interactions are relatively scarce, but their number is increasing rapidly. The emerging picture is that many of the phenomena known for above-ground interactions between plants and insects also occur below ground. Secondary metabolites influence below-ground herbivores, and herbivores can induce changes in secondary metabolites. The first examples of the emission of below-ground herbivore-induced carnivore attractants have recently been published. Feeding by black vine-weevil grubs induces the emission of chemicals that attract entomophagous nematodes from a distance.[173] Moreover, plant–insect interactions on aerial and subterranean plant parts may be cross-linked through systemic changes in plant characteristics.[17,104] As a result, above- and below-ground herbivores may compete, as was recorded for the interaction between the root-feeding rice-water weevil *Lissorhoptrus oryzophilus* and the folivorous fall armyworm *Spodoptera frugiperda*.[159] Such competition is not always reciprocal.[16] Moreover, below-ground herbivory may modify plant characteristics that indirectly influence folivores through carnivorous enemies of the folivores. For instance, root feeding by *Agriotes lineatus* larvae induces a 10-fold increase in the production of foliar extra-floral nectar, which is well known to stimulate visits by carnivorous insects.[183] Experimental studies have shown that below-ground herbivory influences the rate and direction of vegetation succession and plant species richness.[30] Even phenomena that seem to be exclusively above-ground phenomena may have a below-ground aspect. Folivory in tobacco results in increased nicotine concentrations in the leaves as a result of induced nicotine production in the roots.[4]

Above-ground herbivory can influence below-ground symbiosis of plants with symbiotic fungi. Herbivory on *Plantago lanceolata*, a mycotrophic plant, reduced the colonization of the plants by arbuscular mycorrhizae. In turn, colonization by mycorrhizae reduced the amount of leaf damage, and thus there is a symmetrical interaction between the herbivorous insects and symbiotic fungi.[48]

Because induced plant responses can modify herbivore–carnivore interactions above and below ground, insect–plant interactions may affect multitrophic interactions more comprehensively than is usually considered to be the case.[167] In fact, the division between above- and below-ground interactions is highly artificial and results from methodological rather than scientific arguments. Increasing the effort to make connections between the two will be a major and rewarding challenge in the coming years.

10.4 Microorganisms and insect–plant interactions

Microorganisms are widely present in ecosystems, but usually as hidden players. It is likely that microorganisms are commonly involved in insect–plant interactions (see Section 5.5) and there has been an increased interest in their role in recent years (e.g. Refs 8, 34, 76).

Plant pathogenic microorganisms exploit the same resource as herbivorous insects, and many interactions between the two groups are known.[112] Plants have developed induced defences against each group. Just as is found among herbivorous insects, some pathogens facilitate herbivory, and other pathogens interfere with herbivory. For instance, herbivores often induce defences through the octadecanoid pathway (see Chapter 4) and pathogens through the salicylate pathway. These induced defence pathways are known to interact negatively. In tomato plants the octadecanoid pathway results in induced defences against herbivorous chewing insects, and induced susceptibility to plant pathogens, whereas the salicylate pathway has the reverse effect.[152] However, insect herbivores may additionally activate the salicylate pathway and microorganisms may activate the octadecanoid pathway.[47,107,113,186] Thus, various types of interaction can be, and indeed are, found; this is described as cross-talk in the responses to herbivores and pathogens. It has been suggested that activation of the salicylate pathway by herbivores may enable them to attenuate defences against themselves while activating defences against microbial pathogenic competitors.[47,107]

Apart from plant pathogens, plants harbour many microbial symbionts, such as mycorrhizal fungi and root-nodule bacteria, that affect the plant's nutritional value or its level of toxins. Microorganisms are also present as endocellular, or primary, symbionts in herbivorous insects belonging to the Homoptera, Heteroptera, and Coleoptera[42] (see Chapter 5). These microorganisms supply the herbivores with essential nutrients such as certain amino acids. In addition, secondary microbial endosymbionts have been reported recently in aphids[23] and whiteflies,[29] and microbial symbionts have been reported from the gut lumen of other insects such as grasshoppers and caterpillars.[41] Microorganisms in the gut of herbivorous insects may be involved in the digestion of plant food and the production of elicitors that induce plant defence, and their products in the insect's faeces may be exploited as kairomones by parasitoids. For instance, *Klebsiella* bacteria in the faeces of the leek moth *Acrolepiopsis assectella* produce sulphur-containing breakdown products of secondary plant metabolites that are used as kairomone by the parasitoid *Diadromus pulchellus*.[155]

Microorganisms also play a role as pathogens in insect–plant interactions. The role of microbial pathogens of herbivorous insects has been well studied from an applied perspective (i.e. to control insect pests), with a strong emphasis on the microbial pathogen *Bacillus thuringiensis* (see Chapter 13). Plants can influence the dynamics of the interaction between herbivorous insects and their pathogens. For instance, when the polyphagous winter moth *Operophtora brumata* was feeding on oak, it was killed sooner by winter-moth nucleopolyhedrovirus (NPV) and yielded more virus than when feeding on Sitka spruce or heather.[121] Moreover, juvenile mustard leaf beetles (*Phaedon cochleariae*) grow more slowly when feeding on Chinese cabbage leaves infected by the phytopathogenic fungus *Alternaria brassicae* than larvae feeding on healthy leaves, which makes them more susceptible to the entomopathogenic fungus *Metarhizium anisopliae*.[129]

Microorganisms may be important, but mainly hidden, factors affecting the performance of carnivorous insects. So far, little attention has been paid to pathogens of insect predators or parasitoids (but see Ref. 18). However, there is no reason why these insects should not be affected by pathogens and, indeed, there is evidence that microbial pathogens can interfere with plant–carnivore mutualism. For instance, a baculovirus of the parasitoid *Microplitis croceipes* interferes with parasitoid flight ability and therefore its foraging for herbivorous hosts.[62] Infection with the baculovirus results in reduced vigour and wing deformation. A disease of the predatory mite *Phytoseiulus persimilis* interferes with its response to herbivore-induced plant volatiles.[135] Predatory mites that are infected with the pathogen do not discriminate between the volatiles emitted from prey-infested and uninfested plants, and as a consequence are not arrested in a prey patch. Microbial symbionts appear to be widespread in parasitoids and possibly in arthropod predators. *Wolbachia* has been recorded from many parasitoid species[147] and from some predatory mites.[20] *Wolbachia* influence their host's mode of reproduction and thereby its rate of population increase, which affects parasitoid–host or predator–prey population dynamics.

Appreciation of the involvement of microorganisms in insect–plant interactions is increasing rapidly and we expect that this will yield many exciting discoveries in the years to come.

10.5 Vertebrates and insect–plant interactions

Studies on insect–plant interactions usually exclude interactions with vertebrates. However, vertebrate herbivores can have important direct and indirect impacts on insect herbivores. For instance, vertebrates have much more pronounced effects on plant population dynamics than insect herbivores. Vertebrate herbivores can have a large influence on the composition of plant communities and therefore on food composition for insect herbivores,[125] and this can potentially influence insect community composition. Moreover, vertebrate herbivores may exert incidental predation on herbivorous insects. There are many examples in which vertebrate herbivores have negative effects on insect herbivores. For instance, vertebrates can reduce insect abundance, without an effect of species richness,[54,120] or vertebrates can reduce insect abundance indirectly through changes in plant architecture.[49] These asymmetrical effects may impose a selection on herbivorous insects to select locations among and within plants that are least likely to be consumed by vertebrate herbivores.[187,191]

Vertebrates such as insectivorous birds can promote plant growth resulting from predation of leaf-chewing insects,[96] and birds can discriminate from a distance between insect-damaged and control plants based on plant-related cues other than the visual feeding damage of the insects.[94] However, it is interesting to note that some studies show that insects may have a strong negative effect on vertebrate herbivores. For instance, ants that nest in the thorns of *Acacia drepanolobium* significantly improve their host's defence against browsing mammals, which stop feeding almost completely on trees that harbour ants (Fig. 10.9).[143] Plant characteristics such as thorns do not by themselves have such a strong effect on browsing vertebrates, underlining the importance of the ants in anti-vertebrate defence of the tree.

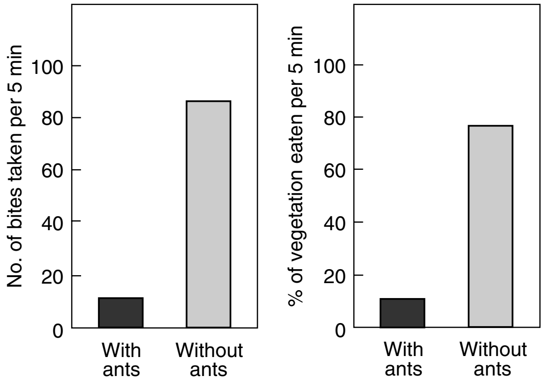

Figure 10.9 Mean number of bites and median percentage of vegetation taken by a goat from *Acacia drepanolobium* trees with or without ants (*Crematogaster* spp.). (Data from Stapley, 1988.)[143]

Vertebrate herbivores affect plants through nutrient cycling. Herbivory may both accelerate and slow down nutrient cycling. Because of their large body size, vertebrates are usually considered to be the most important factor in nutrient cycling. However, insects may accelerate nutrient cycling and consequently plant production as well.[9] It remains unknown how vertebrate and invertebrate effects on nutrient cycling compare or interact. Most likely the effects of insects occur on a smaller spatial scale and with less impact on soil structure than the effects of large, mobile mammalian herbivores that may uproot plants, trample and compact soil.

The interactions between vertebrates and insects on plants is a largely unexplored research field, which is worth investigating because of the potential for important effects.

10.6 Indirect species interactions in communities

Apart from direct physical interactions between organisms, communities are characterized by numerous indirect interactions. These are interactions between two species that are mediated by a third species. For instance, two plant species may share a herbivore that prefers to feed on one of the two plant species. If one of the plant species is tolerant to the herbivore while the other is susceptible, then a population build-up of the herbivore on the tolerant species has a negative effect on the susceptible species, and the two species interact indirectly. There are many examples in which a herbivorous insect influences competition between two plant species. For instance, in a field experiment insecticides were applied to vegetation consisting of the grass *Holcus mollis* and the herb *Galium saxatile*. The treatment removed a grass-feeding aphid, *Hocaphis holci*, and thereby increased the competitiveness of the grass. As a consequence, the abundance of the herb *G. saxatile* decreased.[26]

Direct physical interactions have long been considered the most important interactions in ecosystems. After all, such interactions obviously lead to processes such as interference, consumption, mortality, and reproduction that all have a direct effect on population dynamics. However, it is becoming increasingly clear that indirect interactions are very important as well. For instance, when mayfly larvae in a stream perceive the presence of their predators through infochemicals, they restrict grazing on algae and spend relatively more time hiding to avoid falling victim to their predators. This has a positive effect on algal biomass production.[100] A study of interactions between *Phyllonorycter* leaf-mining moths and their parasitoids indicated a large number of potential indirect interactions. The strongest potential indirect interactions among the leaf-miners were between those species that attack the same host plant because they have the most important overlap in parasitoid species.[130] The strength of indirect interactions is often, on average, weak compared with direct interactions. Effects were identified as 'weak' when the removal or addition of a species did not result in a statistically significant effect on the abundance of the target species.[13] Despite indirect interactions being weak on average, however, the variation in strength of weak interactions may be large—usually larger than the variation in strength of strong interactions—and occasionally their strength may become even larger than the mean value of strong interactions. This means that there are circumstances where weak interactions can be much stronger than strong interactions and, therefore, interactions should not be ignored because they are on average weak.[13]

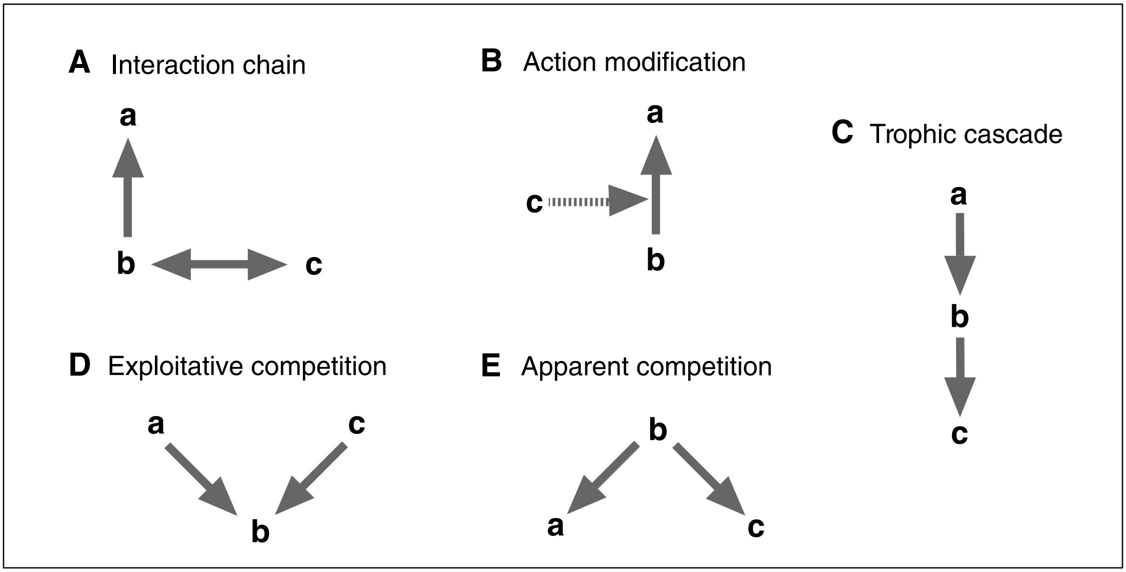

Figure 10.10 Different types of indirect interactions among species. (**A**) Species **c** affects species **a** through a chain of direct interactions involving a change in abundance of species **b**. (**B**) Species **c** affects species **a** indirectly by modifying host species **a** interaction with species **b**. (**C–E**) Three commonly investigated types of simple indirect effects: (**C**) trophic cascade, (**D**) exploitative competition, and (**E**) apparent competition. (Modified from Wootton, 1994.)[188]

Indirect interactions may occur in two major ways: (1) changes in the density of one species affect a second species through its numerical effects on an intermediary species that has a direct effect on the second species (Fig. 10.10A) and (2) an intermediary species modifies the specifics of an interaction between two other species (Fig. 10.10B). Indirect interactions can be mediated by many different traits, such as morphology, chemistry, and behaviour. Several types of indirect interaction have been recognized (see Fig. 10.10C–E).[188] Examples are discussed below.

10.6.1 Exploitative competition

Exploitative competition (Fig. 10.10D) occurs when two organisms compete for the same resource without physically interfering with one another. This has been a central issue in community ecology for a long time. Interspecific competition has long been considered unimportant among herbivorous insects.[149] One of the arguments was that plants commonly have open niches for herbivores. An impression of the number of vacant niches may be obtained by comparing the occurrence of specialized herbivorous insects on the same plant species in different geographical regions. Figure 10.11 shows that bracken fern (*Pteridium aquilinum*) is exploited to different degrees in six geographical regions. This indicates that (1) there are several vacant niches on bracken fern in, for example, a North American habitat compared with bracken in England, and (2) the same resource can be exploited by several insect species (e.g. 10 species

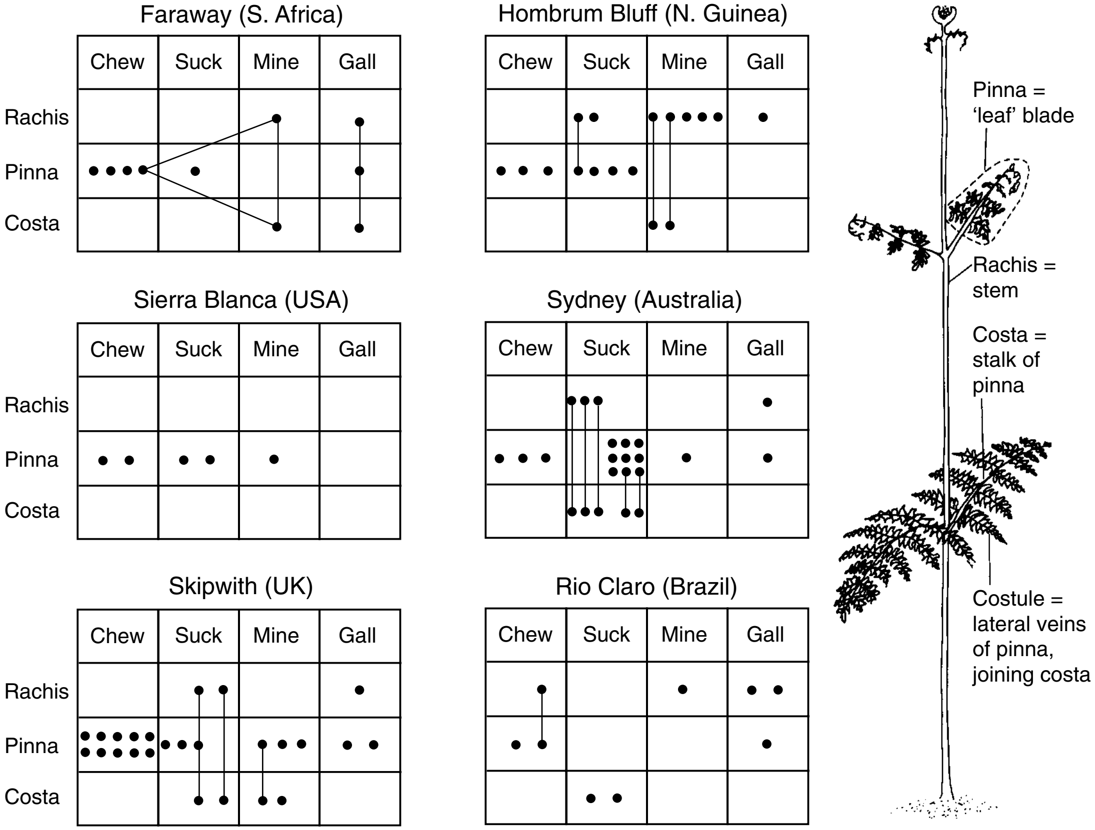

Figure 10.11 Feeding sites and feeding types of herbivorous arthropods attacking bracken fern (*Pteridium aquilinum*) in six different parts of the world. Each dot refers to one arthropod species; feeding sites of insects that exploit more than one plant part are joined by lines. (Redrawn from Lawton *et al.*, 1993.)[89]

in England chew the pinna). Lawton *et al.* concluded that the colonization of bracken fern by herbivorous insects over evolutionary time has been largely a stochastic process that has not been constrained by interspecific interactions between the herbivores.[89] However, more recent evidence indicates that interspecific competition is likely to be much more important among herbivorous insects than was considered to be the case until the 1980s. Two independent meta-analyses showed that interspecific competition is found quite frequently, although its strength and frequency vary considerably among systems.[28,33] In a comparison of studies of 104 plant–herbivore systems, Denno *et al.* reviewed data on 193 potentially competing species pairs.[33] For this meta-analysis, the authors had included those interactions between two herbivorous species for which there was direct evidence for or against competition and facilitation. Three-quarters of the interactions were characterized by interspecific competition. The proportion of competitive interactions was much higher among sap-feeding insects than among free-living leaf-chewers, and among related than non-related taxa. Sap-feeders, stem-borers, wood-borers, and fruit- and seed-feeders had highest proportions of interspecific competition (Table 10.1). The high frequency of interspecific competition reported for seed- and fruit-feeding herbivores may be skewed owing to a relatively large number of laboratory studies (8 of 21 studies on seed- and fruit-feeding herbivores). However, overall, 166 of the 193 cases represent field studies. Therefore, these investigations show that interspecific competition can occur frequently, although we should keep in mind that studies of interactions between species with characteristically low densities are probably under-represented among experimental studies. Inducible defences have systemic effects and may act over long time periods. This indicates that interspecific competition can occur over temporal and spatial scales that were previously not considered. For instance, root-feeding aphids compete with leaf-galling aphid species without direct physical contact.[104]

Table 10.1 Frequency of interspecific competition among herbivorous insects with different feeding styles (based on Denno *et al.*, 1995)[33]

Feeding style	% interactions with interspecific competition	No. of interactions investigated
Sap feeding	88	48
Stem boring	93	14
Wood boring	93	15
Seed and fruit feeding	100	21
External leaf feeding	57	53

10.6.2 Apparent competition

In this type of competition two organisms interact through a shared predator (Fig. 10.10E). The mechanism here is that the density of one resource species affects the density of the consumer, and this subsequently affects the density of a second resource species.[72] For instance, when population densities of an aphid were increased by fertilizing their host plants, this resulted in a decline of neighbouring populations of another aphid species that was caused by an increase in coccinellid predators.[105] Apparent competition has long been considered rare, but the number of examples is increasing rapidly (e.g. Refs 22 and 105). A special case of apparent competition may occur through the presence of alternative food such as pollen for a carnivorous arthropod. For instance, predation of aphids on alfalfa by the coccinellid predator *Coleomegilla maculata* is higher when alfalfa grows next to dandelions. Feeding on dandelion pollen by the coccinellid results in a higher population density of this predator and, consequently, a lower aphid density on alfalfa (Fig. 10.12).[63] Analogously, apparent competition can occur between plant species as a result of interactions with a shared herbivore species.[157]

10.6.3 Trophic cascades

Trophic cascades (Fig. 10.10C) are a focal point of food-web dynamics studies and a popular example of indirect effects. In a trophic cascade, one trophic interaction has effects on a subsequent trophic interaction in a food chain, so that a trophic cascade occurs when changes in carnivore abundance alter the distribution and abundance of plants.[110,115,132]

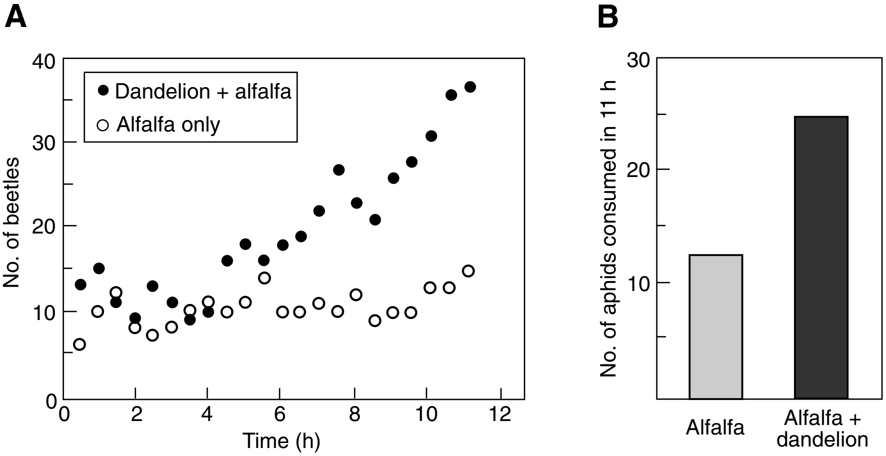

Figure 10.12 (**A**) Number of *Coleomegilla maculata* beetles (Coleoptera: Coccinellidae) observed in cages either on the side with alfalfa plants infested with aphids or on the side with alfalfa plants infested with aphids that were mixed with dandelion plants without aphids. (**B**) Number of aphids consumed during the experiments on alfalfa plants at either side of the cage. (From Harmon *et al.*, 2000.)[63]

Trophic cascades provided the basis for the 'world is green' hypothesis, which states that predator effects on populations of herbivorous insects result in an abundant plant survival with the result that the world is green.[58] However, whether a trophic cascade occurs is dependent on the relative strength of carnivore–herbivore and herbivore–plant interactions, as well as the number of connections in a food web. The more linear the food web, the stronger the effect of removal of carnivores on the abundance of plants. With an increase in reticulateness, the removal of a carnivore may be compensated for by modifications in other trophic interactions in the food web. The occurrence of trophic cascades has been hotly debated in the ecological literature. Trophic cascades have been amply demonstrated for aquatic systems, but were considered much less important in terrestrial systems.[114,150] The main arguments for this were the following. Anti-herbivore defences in terrestrial plants weaken the relative effect of herbivores on plants compared with those of carnivores on herbivores. Furthermore, trophic cascades are thought to be restricted to communities with low species diversity where a restricted number of species dominate the community. Terrestrial ecosystems are characterized by highly reticulate food webs, and as a result the effect of a single species will be distributed through different connections in the food web.[150] However, a recent review has indicated that trophic cascades are likely to be more common than often considered. Schmitz *et al.* carried out a meta-analysis of 41 studies on trophic cascades in terrestrial systems, reporting 60 independent tests. In all cases the herbivores were arthropods.[132] These authors concluded that trophic cascades were common: carnivore removal affected plant variables such as damage, biomass, and reproduction in 45 of the 60 tests. Carnivore effects were stronger when the plant response was measured in terms of damage rather than plant biomass or plant reproduction. Effects on plant damage do not always translate into effects on plant reproduction, because plants may compensate for damage and/or show tolerance to herbivore damage. In several cases an attenuation of effects was observed when the effects on herbivores were compared with those on plants: the direct effects of carnivores on herbivores were stronger than the indirect effects on plants. Some of these cases could be explained by some form of anti-herbivore defence of plants. The suggestion that more complex food webs dampen the strength of trophic cascades was supported by the meta-analysis of Schmitz *et al.*[132] In conclusion, current knowledge indicates that trophic cascades may be common, but the importance of their effects still needs to be established, especially in field studies on an appropriate time-scale.[73] This also relates to the debate on the importance of small effects on community processes (see Section 10.7).[98]

10.7 Species interactions and phenotypic plasticity

Species interactions are often assumed to be fixed: all organisms of a population have the same characteristics and interact in the same way with other organisms. However, organisms are phenotypically plastic, that is, they can express different phenotypes depending on the biotic and abiotic environment.[2] Phenotypic plasticity occurs in species at all trophic levels. For instance, feeding by herbivorous insects can induce a multitude of chemical and physical changes in their food plant and these phenotypic changes can be dependent on the herbivore species that inflicts the damage (see Chapter 4). Induced plant responses affect interactions between the plant and other herbivore species, between the plant and carnivores, and among competing plants.[36] For instance, the induction of defences in wild tobacco plants by feeding *Manduca* caterpillars has severe costs to the plant that are manifested in the competition for resources with uninduced neighbouring conspecific plants. The induced plant grows much more slowly than its uninduced neighbour and produces fewer seed capsules. Interestingly, induced plants that compete among one another grow as rapidly and produce as many seeds as uninduced plants that compete amongst each other (Fig. 10.13).[165] The mechanism of this remains to be elucidated, but the data show that undamaged tobacco plants have an opportunity benefit when growing next to an induced neighbour.

Phenotypic changes in herbivorous insects can be caused by plants. The searching behaviour of herbivores is influenced by community composition. Herbivores can develop a search image for certain host plants, dependent on their experiences during foraging behaviour, and this results in different host selection behaviours in different environments (see Chapter 8).[111] For instance, the response of tephritid fruit flies to a marking pheromone can-depend on fruit size and the density of alternative fruits.[119]

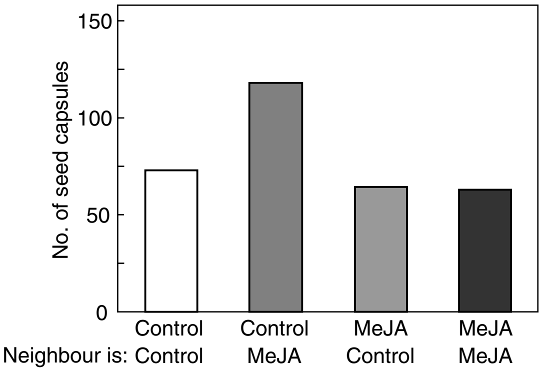

Figure 10.13 Number of filled seed capsules produced by *Nicotiana attenuata* plants that had different treatments and differently treated neighbours. Plants were either treated with methyl jasmonate (MeJA) to induce defences or treated with an appropriate control. Control plants that competed with induced plants had a significantly higher rate of reproduction than plants from any other treatment. (From Van Dam and Baldwin, 1998.)[165]

Phenotypic changes in herbivorous insects can also be induced by carnivorous insects. The presence of carnivores can result in behavioural changes such as avoidance behaviour or shifts in time allocation to foraging versus hiding, as well as in morphological changes.[38] For instance, the spotted cucumber beetle *Diabrotica undecimpunctata howardi* reduced feeding when exposed to chemical information from the wolf spider *Hogna helluo*, but not when exposed to information from three species of other, less dangerous, predators.[139]

Carnivorous insects exhibit different characteristics in response to changes in resource availability[176] and natural enemies.[71] Previous experience can influence the foraging behaviour of carnivorous insects during various phases.[176] For instance, parasitoids can associatively learn to respond to herbivore-induced plant volatiles and consequently temporally restrict their foraging behaviour to a specific plant species.[162]

As a result of phenotypic plasticity, interactions in an ecosystem are context dependent, and phenomena recorded for one ecosystem are not necessarily found in other ecosystems. Context dependency implies that interactions are influenced not only by genotype but also by the physiological and informational state of the insect, and the state of resources and natural enemies.[119]

10.8 Top-down versus bottom-up forces

It has been long and intensively debated whether communities are shaped by top-down (consumer-driven) or bottom-up (producer-driven) forces.[74,185] This also relates to the discussion on the most important factors that influence the effects of plant diversity on herbivorous insects, especially in the context of monocultures versus polycultures in agriculture. In a monoculture the abundance of a specialist herbivore per individual host plant is usually much higher than in a polyculture of the same plant species (Fig. 10.14). The resource concentration hypothesis stresses bottom-up forces and proposes that herbivores are more likely to find and remain on hosts that are growing in dense or nearly pure stands, and that the most specialized species frequently attain higher relative densities in simple environments.[127] The enemy hypothesis stresses top-down forces and predicts that there will be a greater abundance and diversity of entomophages in more diverse plant communities, resulting in reduced numbers of herbivorous insects.[127]

In an influential paper by Hairston and colleagues, the observation that 'the world is green' despite a large number of species and individuals of herbivorous insects has been explained by the effects of carnivorous enemies that regulate populations of herbivorous insects.[58] However, others have supported the importance of bottom-up forces. For instance, induced plant responses were reported as the main cause for cycles in the population dynamics of the autumnal moth *Epirrita autumnata*,[64] and a model of induced resistance (see Chapter 4 for the difference between defence and resistance) shows that such a bottom-up force can both regulate and drive persistent fluctuations in herbivore populations in the absence of other density-dependent factors.[164] In the past 10 years it has become more and more clear that, rather than two mutually exclusive forces, top-down and bottom-up forces are often intimately connected. This emerging concept runs parallel to the interest in the effects of indirect interactions, which also indicate that top-down effects can influence bottom-up effects and vice versa.[1,72,188] For instance, bottom-up forces such as plant quality can affect the composition of insect communities and this can subsequently influence top-down forces. Furthermore, plants have many characteristics that influence the effectiveness of carnivores in reducing herbivore numbers.[35,116] For instance, plant characteristics such as the presence of domatia or herbivore-induced volatiles can increase the effects of carnivores on herbivores. The discussion, therefore, is changing from which of the two forces is shaping communities towards what the relative contribution of the two forces is. Moreover, both top-down and bottom-up forces are subject to variation, which can be important in determining their effects on herbivorous insects.[55,79,126] For instance, herbivorous insects should decide whether to accept a currently encountered host plant for oviposition or to search for a better one. Which is the best decision depends on the density and distribution of host plants, as well as the variation in their quality on the one hand and the distribution of carnivores and the variation in the mortality risks that they impose on the other. For example, when the probability of a catastrophe such as complete consumption of a host plant by a browsing mammal is large, the pay-offs for spreading your offspring over more host individuals may increase, even when these plants are of a lower quality.[126]

One topic within the top-down and bottom-up debate that relates particularly to herbivorous insects is why most of them are specialist

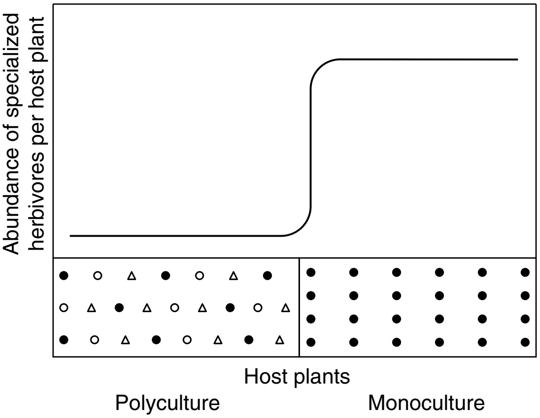

Figure 10.14 Effect of polyculture versus monoculture on the abundance per host plant of specialized herbivores on the plant species represented by black dots. (From Strong *et al.*, 1984.)[149]

feeders.[14,75,134] For a long time it was assumed that this could be explained by plant chemistry. Plants are highly diverse in secondary metabolites and insects could cope with this daunting complexity only by specializing on a restricted number of related host-plant species with a restricted number of secondary metabolites. In a seminal paper, Bernays and Graham provided evidence for an important role of carnivores as well.[14] Specialist herbivores are better protected against their natural enemies and make quicker decisions on where to feed. Quick decisions on acceptance of a host plant and short feeding durations can be important in reducing the odds of falling victim to a predator or parasitoid.[15] The importance of (avoiding) interactions with natural enemies during foraging behaviour of herbivorous insects has received increasing support. For instance, *Pieris* butterflies prefer a suboptimal food plant that is relatively enemy free over a nutritionally superior host plant on which parasitoids are an important source of mortality,[108] and a similar phenomenon has been recorded for the leaf beetle *Oreina elongata*.[6] It is interesting that these studies have been conducted under field conditions.

It has become clear that food-plant choices of herbivorous insects may have consequences in terms of food quality and risks of attack from natural enemies (see also Section 11.7). Therefore, top-down and bottom-up forces should not be seen as alternatives, but rather as two complementary forces. Rather than studying whether one or the other force is in operation, it is more informative to investigate the relative contribution of the two forces in different ecological systems.

10.9 Food webs and infochemical webs

Species in ecosystems are linked through consumer–resource connections as well as through connections that are mediated by chemical information. Food webs comprise the trophic relationships between sets of interacting species, whereas the sets of relationships that are mediated through chemical information are called infochemical webs. A food web is overlaid by an infochemical web that has more connections than the food web, because organisms that do not have a trophic interaction may well be connected through infochemicals.

10.9.1 Food webs

The comprehensive analysis of a complete food web provides major problems because of the large numbers of species involved. Therefore, most food-web analyses are restricted to a subset of strongly interacting species. Three main types of food webs can be distinguished: (1) connectance webs that present trophic links without quantitative information on the relative frequencies of the trophic links, (2) semiquantitative webs that include information on the relative abundance of trophic links, and (3) quantitative webs that express all trophic links in the same absolute units.[130] To illustrate the complexity of quantitative food webs, consider the food web of *Phyllonorycter* species (Lepidoptera: Gracillariidae) and their parasitoids on four plant species (Fig. 10.15).[130] Over a 2-year period 12 *Phyllonorycter* species were reared from four tree species. These herbivores were attacked by a total of 27 parasitoid species. The structure of the food web was strongly influenced by the host plant. It should be realized that a food web is a 'snapshot of community dynamics' that varies in time, for instance because of non-synchronous population dynamics and because the number of generations per species varies. Thus, there is temporal variation in food-web dynamics. The total estimated number of *Phyllonorycter* individuals in the study area (10 000 m^2) over the two years was about 75 million. Of the 324 (12 × 27) possible parasitoid–host interactions 132 (41%) were observed, whereas of the 48 (12 × 4) possible herbivore–plant interactions only 12 (25%) occurred.[130] It should be realized that, despite the major effort that was made to construct this food web, it is only a small part of the local community. Not included are, for example: (1) predators and pathogens of the *Phyllonorycter* herbivores, (2) other herbivore species and their parasitoids, predators, and pathogens, and (3) other plant species and their herbivores plus parasitoids, predators, and pathogens. Other food webs have included the fourth trophic level—hyperparasitoids.[106] So far, plant–pollinator food webs have been studied

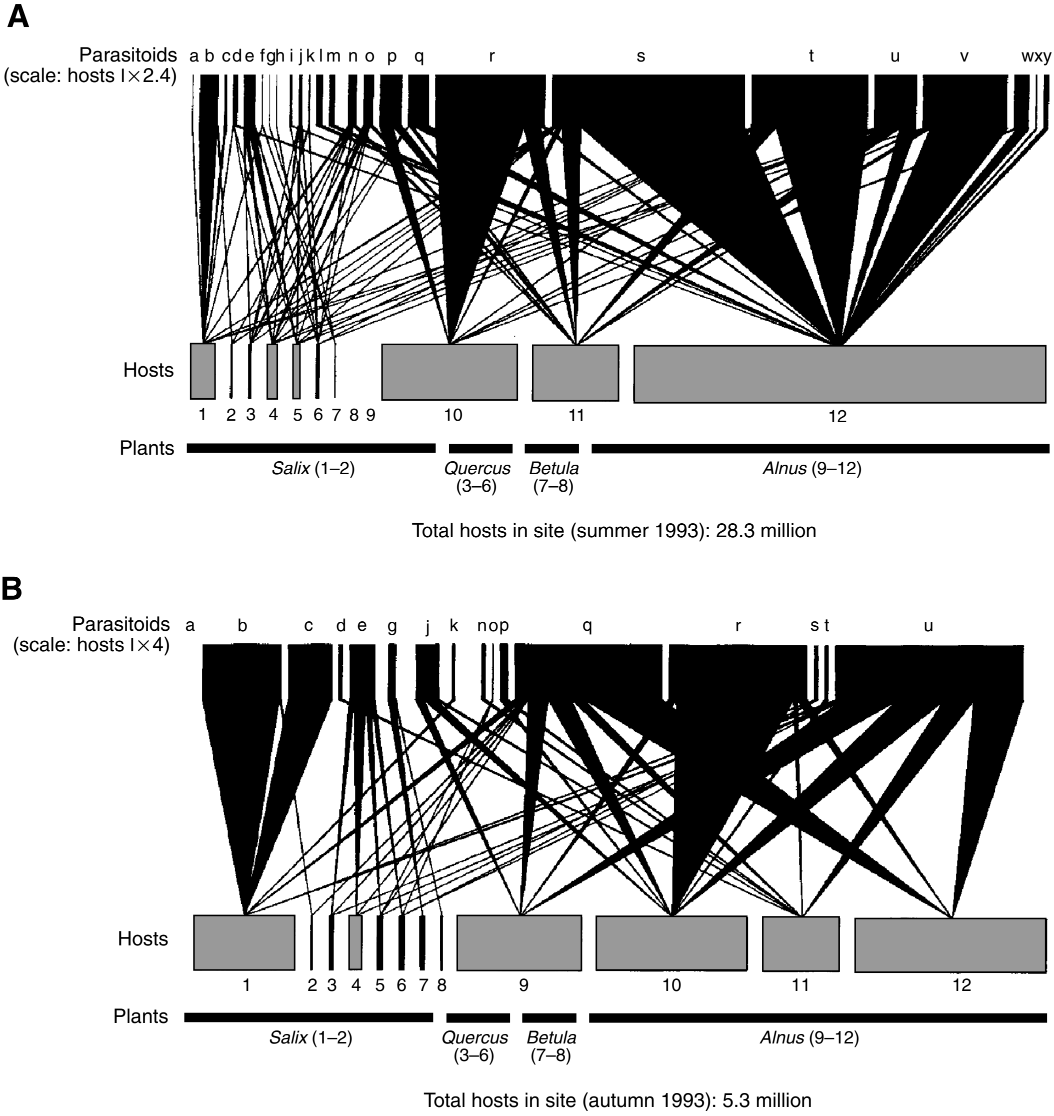

Figure 10.15 Quantitative plant–host–parasitoid webs for two generations of leaf-mining moths in the genus *Phyllonorycter* (Gracillariidae) and their parasitoids at a site in southern England. Twelve species of *Phyllonorycter* (coded 1–12) were reared from four tree species. The moths were attacked by 27 species (a–y) of hymenopterous parasitoids. Numbers between brackets are the code numbers of the insect species that feed on that tree. The widths of the bars representing the tree, herbivore, and parasitoid species are proportional to the estimated total number of individuals in the site, so that parasitoid species s, *Phyllonorycter* species 12, and *Alnus* trees were the most abundant species in the summer of 1993. (Modified from Rott and Godfray, 2000.)[130]

separately (e.g. Ref 101), but direct and indirect interactions between herbivores or carnivores and pollinators can occur as well.[43,148]

In a source-based connectance web describing the trophic interactions among herbivores, parasitoids, predators, and pathogens associated with broom in southern England, a total of 154 taxa were recorded with 370 trophic links. This connectance web does not provide details on the frequency of the trophic links. However, it does provide data on a range of herbivore species with different types of their enemies, showing that there is a higher connectance (observed number of trophic links divided by the maximum possible) within the predator sub-web compared with the parasitoid sub-web. Predators consumed significantly more species than did parasitoids.

Quantitative food-web analysis can be used to obtain information on potential direct and indirect interactions that connect species in a community. Moreover, it can be used for comparative analyses. For instance, Omacini *et al*. compared an aphid–parasitoid web on Italian ryegrass that was grown from either endophyte-free or endophyte (*Neotyphodium*)-infected seed.[109] They showed that the presence of the endophyte affected relative aphid abundance and subsequently influenced food-web complexity. On endophyte-free plants, complexity was greater due to an increased number of trophic interactions per species, and the number of indirect links through shared parasitoids.

10.9.2 Infochemical webs

Every member of a food web produces infochemicals that can influence direct interactions. Consumers exploit infochemicals from their resources, and resources can exploit infochemicals from their consumers. For instance, herbivorous insects use plant volatiles to locate their food plants[36,178] (see Chapter 6), as well as infochemicals from their enemies to avoid becoming a meal.[38] Moreover, an infochemical that is released into the environment can be exploited by any organism of the community to meet its own needs. As a result, infochemicals mediate ample indirect interactions. For instance, herbivore-induced plant volatiles may repel or attract herbivores, but they indirectly affect carnivore–herbivore interactions through the attraction of carnivores as well. Differential responses by different carnivore species may mediate the degree to which they compete for the same resource or interact through intra-guild predation. Carnivorous arthropods largely rely on herbivore-induced plant volatiles in locating herbivores or their microhabitat from a distance. Herbivore-produced infochemicals play a role only at short distances. Moreover, herbivore-induced plant volatiles can affect herbivore–plant and carnivore–herbivore interactions on neighbouring plants through their effect on the neighbour's phenotype.[37]

Infochemicals affect virtually all types of behaviour in a community and one behaviour can be influenced by a range of infochemicals from different sources. For instance, an ovipositing herbivore can use chemical information on the food plant's identity and quality,[169] on the presence of competitors,[133] on the presence of herbivores that provide protection,[137] and on the presence of natural enemies.[38] Optimal foraging theory assumes that animals are omniscient and that this allows them to make 'decisions' that maximize fitness.[145] Although omniscience of insects has often been considered unrealistic, and although there will always be constraints on the information available to an insect, there is increasing evidence that insects are remarkably well informed about prevailing conditions.[84,177] As a consequence, when insects do not seem to make the best decision, this may be explained by more than a 'mistake'. Sometimes, we may not be aware of the information on which the insect bases its 'decision'. For instance, adult insect herbivores can choose oviposition sites that enhance their own long-term fitness at the expense of the fitness of their individual offspring. This suggests that herbivorous insects might be genuinely bad mothers, but that host choice is nonetheless adaptive, and that theory needs to incorporate new assumptions about host effects on adult performance.[97]

In summary, food webs are overlaid by a highly reticulate infochemical web[35,136] that is affected by and affects trophic interactions, as shown by the example of the effects of herbivore-induced plant volatiles (Fig. 10.16).

Infochemical emission by an organism not only changes its phenotype from, for example, an inconspicuous to an apparent one, but through its multiple effects on members of the community it can also change food-web interactions and community composition.[181] However, to date, infochemically mediated interactions have been investigated exclusively for isolated interactions within food webs.

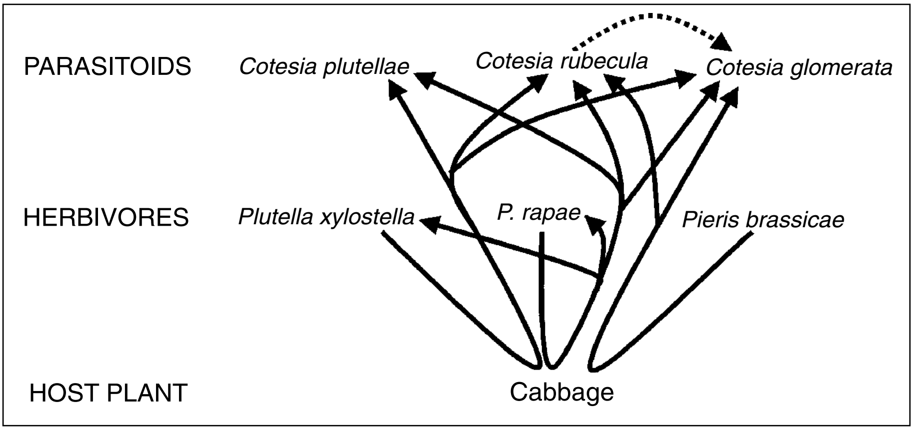

Figure 10.16 Infochemical web. Infestation of cabbage plants by caterpillars of different herbivorous species results in attraction of parasitoids and also influences oviposition preferences of adult herbivores. (Based on data from Shiojiri *et al.*, 2001, 2002;[136,137] Geervliet *et al.*, 1994, 1998.[51,52])

10.10 Communities

Communities are groups of species that interact or have the potential to do so. As we have seen above, species interactions extend beyond direct interactions. In fact, when considering communities of insects and plants, we cannot restrict ourselves to these major taxa. Plants, as well as insects, interact with other taxa ranging from microorganisms to mammals. An interesting example is that of the connection between acorns, gypsy moths, mice, deer, ticks, and Lyme disease.[77] Oak trees produce large amounts of acorns once every 2–5 years and acorns are a critical food for white-footed mice in the eastern USA. These mice are also important predators of pupae of the gypsy moth *Lymantria dispar*. The gypsy moth can experience outbreaks resulting in defoliation of oaks over large areas and thus this insect has considerable impact on oak trees. Large amounts of acorns result in white-tailed deer moving into oak forests and these deer, together with the white-footed mice, are the primary host of the black-legged tick *Ixodes scapularis*. The tick is a vector of spirochaete bacteria, which cause Lyme disease in humans. Ticks drop from the deer and reproduce. Their offspring become infected with the bacteria on mice. Acorn density affected mice and deer, and consequently both gypsy moth outbreaks and disease transmission by ticks.

When in southern England rabbit populations, which maintained a close-cropped turf, were decimated by myxomatosis, the turf grew dense and tall. As a result the cooling of the soil that occurs when a short sward grows just a few centimetres taller caused a rapid decline of the ant *Myrmica sabuleti*, which hosts the larvae of the large blue butterfly *Maculinea arion*. This resulted in the 1950s in the extinction of this beautiful rare insect in the UK.[158] These examples show how direct and indirect interactions among taxonomically diverse species link plants to various community members.

Despite these exciting examples, most studies of insect–plant communities have been restricted to plants and insects. Major issues in community ecology are to understand community composition and dynamics. The composition and dynamics of communities are determined by colonization, extinction, and species interactions. Therefore, it is a challenge for future studies to investigate how interactions between insects and plants affect other members of communities. A first step when investigating interactions between plants and herbivorous insects is the incorporation of members of a new trophic level. Members of the third trophic level may comprise birds or vertebrates, in addition to carnivorous insects such as predators and parasitoids.

10.10.1 Why are so many herbivorous insect species 'rare'?

It is common knowledge among taxonomic entomologists that in almost all higher insect taxa (genera, families) many or even most species are 'rare', that is, they are difficult to find or absent in localities and periods where and when they might be expected to be present given their life history, host-plant availability, etc.[50] An explanation can be sought in the hypothesis that herbivores are seldom food-limited but appear most often to be enemy-limited.[58] This has been supported by, for instance, Root and Cappuccino, in their 6-year study of the herbivorous insect assemblage associated with a natural goldenrod (*Solidago altissima*) population: of the 138 species only seven were abundant and even these seldom reached densities at which they caused a decrease in the density of the remaining species.[128] A further explanation could be that host-plant quality is not optimal for herbivorous insects (see Chapter 5 for a detailed discussion of this concept). According to Wratten, this fully explains why the density of herbivores is generally much lower than the available food resources could support.[189] Extreme weather conditions may be a further partial explanation for the 'rarity' of many species and the cause of temporary local extinctions, such as that observed for *Euphydrias* butterflies.[44] Even if one supposes that 'rarity' results from the combined action of the above-mentioned factors, an intriguing question remains unanswered: Why are some insect species abundant and why are other (often closely related) species 'rare'? However, the 'rarity' of many herbivorous insect species is a fact that should be considered thoroughly before generalizations are made concerning the ecology and evolution of insect–insect and insect–plant interactions.[50]

10.10.2 Colonization

On a geographical scale the number of insect species per plant is affected by the area covered by the plant. Geographically widespread plant species generally have more insect species feeding on them than similar but less widespread plants (Fig. 10.17). On a smaller scale, the colonization of a plant population is affected by its size. In addition, its isolation from insect sources and the size of the insect source populations are important determinants of colonization. Theoretical models such as the equilibrium theory of island biogeography predict the number of species on islands as a function of island size and isolation. This relates both to real islands as well as to habitat patches surrounded by unhabitable patches.[53] In small patches with a smaller number of species there is a loss of ecosystem functions, such as decomposition, pollination, parasitism, and predation.[144] Furthermore, ectophagous and endophagous (more specialized) insects are expected to be differentially affected.

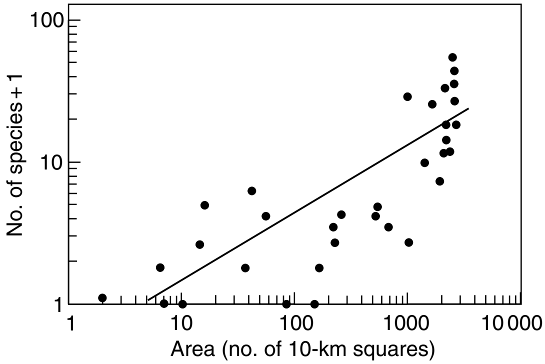

Figure 10.17 Number of herbivorous insect species associated with perennial herbs in Britain as a function of the plant's geographical range. (Modified from Lawton and Schröder, 1977.)[88]

In a field study on bush vetch (*Vicia sepium*), the area of the meadows in which the vetch plants occurred was the major determinant of species diversity and population abundance of endophagous insects in the vetch pods.[86] In a separate experiment with isolated potted vetch plants that were placed at different distances from the meadows, colonization success greatly decreased with increasing isolation (i.e. distance from the meadow). Effects on parasitoids were stronger than effects on herbivorous insects (Fig. 10.18). As a consequence, the percentage parasitism of herbivores significantly decreased with area loss and increasing isolation.[86] Similar results were also found on a smaller scale (only 12 metres) for parasitism of pollen beetles in oilseed rape.[156]

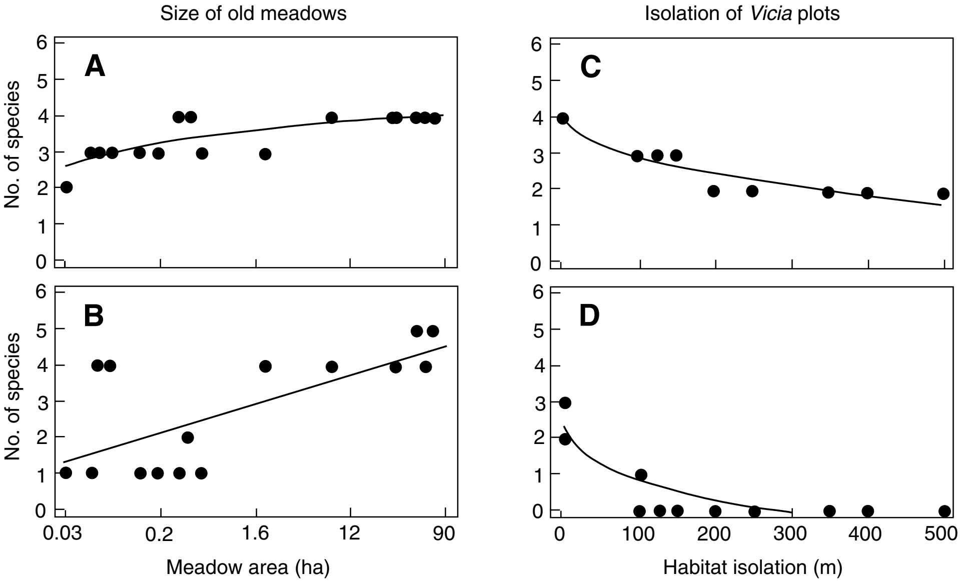

Figure 10.18 (**A, B**) Dependence of species richness on the habitat size of old meadows. (**A**) Number of phytophagous insect species; (**B**) number of parasitoid species. Comparison of regression lines of phytophagous and parasitoid species showed significant differences in both slope and intercept. (**C, D**) Dependence of species richness on the isolation of small *Vicia* plots. (**C**) Number of phytophagous insect species; (**D**) number of parasitoid species. Comparison of regression lines of phytophagous and parasitoid species showed no difference in the slopes but significant differences in the intercepts. (From Kruess and Tscharntke, 2000.)[86]

In conclusion, landscape structure can decisively influence local species composition and species interactions in communities.

10.10.3 Community development

To analyse the development of communities, two types of experimental study can be made: defaunation by the use of insecticides, or the introduction of plants to new regions. The first of these involves a major effort and has been carried out by Simberloff and Wilson.[138] The second type of experiment has been done many times unintentionally: humans have introduced many plant species into new regions and thereby exposed them to new pools of insect populations. This has enabled the study of community development on novel host plants (Fig. 10.19). For instance, in a food-web study on broom plants, the community composition in two native and two exotic habitats was investigated.[102] In the exotic habitats generalist herbivores were dominant, and in native habitats specialist herbivores were dominant. There were no differences in the average abundance of generalist herbivore species per plant between native and exotic habitats. In the exotic habitat empty niches were found; for instance, flower- and seed-feeding herbivores were absent. This suggests

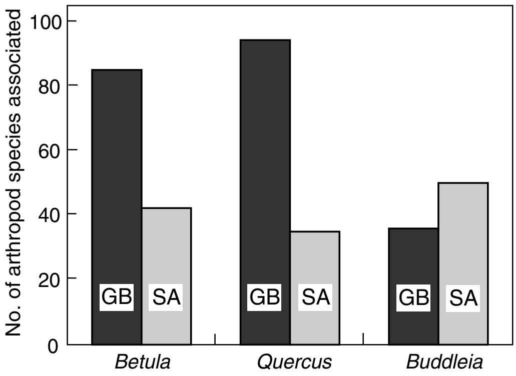

Figure 10.19 Three tree species, *Betula pendula*, *Quercus robur*, and *Buddleia* spp., are found both in Britain (GB) and in South Africa (SA). *Betula* and *Quercus* are native to Britain but were introduced to South Africa, whereas for *Buddleia* the converse is true. The species richness of herbivorous arthropods associated with the trees is clearly less for introduced trees compared with native trees. (Redrawn from Southwood *et al.*, 1982.)[140]

that generalists are the first to colonize a newly introduced plant species, whereas specialists take longer. With increasing herbivore biomass, natural enemies (predators and parasitoids) increased, indicating that plants first accumulate herbivores, subsequently followed by carnivores. This pattern of community development has been reported for many other introduced plant species.[149]

The community development is a stochastic process that is dependent on local species pools and environmental conditions. Moreover, different existing communities may provide different ecological opportunities for insects to colonize novel plant species, for example through the close proximity of widespread and abundant normal host plants to the new host plant. This has been demonstrated in a laboratory study on the colonization of cucumber plants by spider mites. The abundant availability of the suitable host plant lima bean enhanced the colonization of the initially unsuitable host plant cucumber as a result of rapid adaptation of the herbivores.[56] Furthermore, the plant community that is invaded by an exotic plant species can influence the exotic species through the herbivore community. The larger the number of ecological niches for herbivores, which is a function of plant diversity, the more difficult it is for an exotic plant species to invade a plant community. Moreover, the specific species composition of the local plant community can be important, because of shared herbivore species.[118] As a result of such different conditions, community composition and development may vary between different regions.[161]

The establishment of exotic insect herbivores depends partly on the degree of interspecific competition. Although this was considered to be relatively unimportant, recent reviews have demonstrated that interspecific competition is more important than often thought, especially among sucking herbivores.[28,33] It is interesting that an extreme form of interspecific competition (i.e. competitive displacement) was recorded for exotic arthropod species: 37 of 48 reported cases of displacement were caused by exotic species.[122] In addition to competition, the degree of food specialization and the impact of natural enemies strongly influence the establishment of exotic insect species. In general, invasive insect species are characterized by polyphagy and a lack of natural enemies.

10.11 Molecular ecology

Ecologists increasingly adopt novel molecular methodology to address questions that are otherwise difficult or impossible to address. Molecular approaches have opened many new exciting avenues to the field of insect–plant interactions. After many years in which biochemists and molecular biologists on the one hand and ecologists on the other worked in isolation on insect–plant interactions, this apartheid is breaking down and integrated approaches are currently being made. For instance, there is increasing knowledge of the molecular mechanisms of induced plant defences against herbivorous insects. A range of genes involved in such plant defences has been characterized and novel cDNA microarray technology allows for the monitoring of gene expression choreography as induced by insect herbivory.[69,123,124] Some initial information has indicated that *Manduca sexta* feeding on tobacco results in the upregulation of many genes involved in response to stress, wounding, and pathogens, as well as the genes involved in allocating carbon and nitrogen to defence. This fits well with the ecological theory of a trade-off between growth and defence for plants.[68] Comparison of the effects of mechanical wounding versus insect herbivory can illuminate which genes are specifically upregulated or downregulated in response to herbivory, and this may indicate whether the herbivore manipulates plant defences or whether the plant exploits herbivore elicitors to mobilize specific defences. For instance, herbivory by *Pieris rapae* on *Arabidopsis thaliana* does not induce a range of genes that are upregulated by mechanical wounding and that are involved in plant defences (Fig. 10.20).[123] This suggests that this specialist herbivore manipulates plant defence, a phenomenon also recorded for the specialist *Manduca sexta* feeding on wild tobacco.[78] Furthermore, the cDNA microarray technology can be combined with the use of specific mutant and transgenic plants to investigate the effect of a single mutation or gene modification on the expression

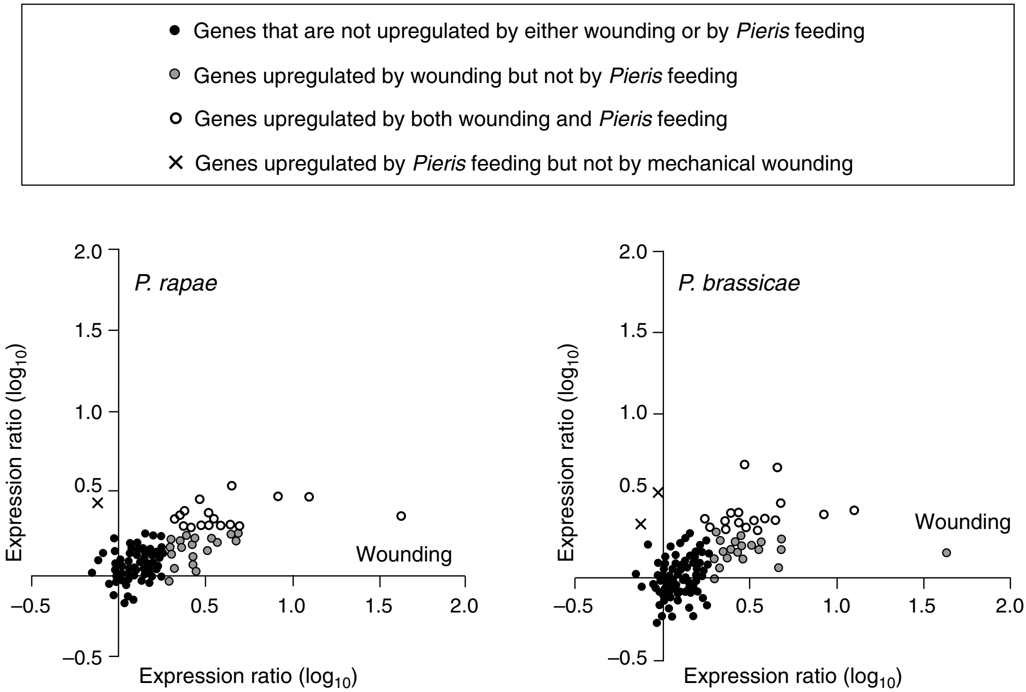

Figure 10.20 Expression levels of 150 genes of *Arabidopsis thaliana* after mechanical wounding compared with feeding damage by *Pieris rapae* or *Pieris brassicae*. (From Reymond *et al*., 2000,[123] and http://www.unil.ch/ibpv/WWWPR/Docs/rapae-brassicae; © American Society of Plant Biologists, with permission.)

profiling of the total genome.[123] Gene expression can be seen as the first step in a plant's response to changing conditions. Therefore, gene expression through Northern blotting or microarrays has been used to investigate plant responses to volatiles from neighbouring plants,[3] to herbivory by specialist versus generalist herbivores,[124,180] to different nutrient levels,[90] to a range of different types of attacker such as different herbivorous insects[65] or herbivorous insects versus pathogenic microorganisms, and to mechanical damage versus herbivore-derived elicitors.[59] This provides initial information on whether and how plants respond to certain treatments and whether they respond differently to different types of treatment. This will lead on to investigation of the contribution of gene expression patterns to phenotypic expression and to interactions with community members. For instance, herbivory by the tobacco hornworm *Manduca sexta* on wild tobacco plants induces the jasmonate signalling pathway. Genes in this pathway are upregulated. By silencing three genes in the jasmonate signalling pathway (i.e. lipoxygenase, hydroperoxide lyase and allene oxide synthase), the importance of these genes in induced defences of the tobacco plants can be investigated in the laboratory[60] and the field.[82] This reveals that lipoxygenase gene activity in particular is necessary for induced defence. When this gene is silenced, the plants experiences a large increase in herbivore damage inflicted both by adapted herbivores such as the tobacco hornworm and by herbivores that do not feed on wild-type tobacco plants such as *Empoasca* leaf-hoppers.[82]

Extensive knowledge exists on the ecology of insect–crucifer interactions, especially related to *Brassica* species.[24,127,136] *Arabidopsis thaliana*, whose full genome has been sequenced, is an important model for plant sciences, and potentially also for ecological research.[103] It is surprising that studies of insect–*Arabidopsis* interactions have so far been limited. However, initial studies show that this crucifer can greatly benefit ecological studies. For instance, *Arabidopsis* responds to insect herbivory in a similar way as other crucifer plants:[171] insect herbivory induces direct[99,146] and indirect,[168,170] defences, and the signal transduction pathways involved are similar to those involved in induced defences in other (cruciferous) plants.[39] *Arabidopsis* provides excellent opportunities for the investigation of individual plant characteristics because of the availability of mutants and transgenic genotypes that differ in a single or restricted number of well characterized genetic modifications. This allows a comparative ecological approach that could only be dreamed of until recently. Such approaches have also been developed for other plant species, such as the wild tobacco *Nicotiana attenuata*.[5] The development of novel methods to silence genes in organisms for which large numbers of mutants are not available is providing a breakthrough that will lead to major advances in our understanding of the role of specific genes in the ecology of organisms.[40,82] Field studies that expose carefully designed treatments to natural conditions are an important component of this approach.[82]

Secondary plant metabolites play an important role in insect–plant interactions (see Chapter 5) and the diverse class of cytochrome P450 monooxygenases (P450s) is involved in both biosynthesis by plants and the detoxification by insects.[11] More than 280 P450 genes have been reported for the genome of *A. thaliana*, and 90 for the genome of *Drosophila melanogaster*. Several P450s are involved in the biosynthesis of furanocoumarins by plants. The lepidopteran *Papilio polyxenes* detoxifies furanocoumarins in its diet by means of at least two P450s whose genes are activated by exposure of caterpillars to the furanocoumarins. Their expression is tissue specific, restricted to the mid-gut and to a lesser extent to the fat body. The induction is mediated by furanocoumarin-responsive elements on the genes.[12] It is remarkable that this element is also present in the related *P. canadensis*, which rarely, if ever, encounters furanocoumarins in its diet. One possible explanation for this is that *P. canadensis* uses similar P450s for the detoxification of other secondary plant metabolites and that the genes can be induced by different compounds.[12]

Molecular techniques can be used for genetic characterization of populations of herbivorous insects to provide a genetic basis for the adaptation of insects to plant defences. This has been applied to studies of the flea beetle *Phyllotreta nemorum* and the crucifer *Barbarea vulgaris* to assess substructuring of the populations. Genetic differentiation was recorded between (sub)populations using spatially separated plant patches at a distance of approximately 100 m to 1 km, and between localities approximately 44 km apart. Genetic differentiation was associated with geographical distance.[31]

A molecular ecological approach can be used to generate new genotypes to investigate the effect of plant traits, such as secondary plant metabolites. For instance, the three-gene pathway for the synthesis of the cyanogenic glycoside, dhurrin, has been transferred from *Sorghum bicolor* to *A. thaliana*. This resulted in decreased flea beetle (*P. nemorum*) feeding damage, a reduced number of mines, and a higher larval mortality rate compared with control *A. thaliana* plants, demonstrating that cyanogenic glycosides confer resistance to herbivores that have not yet evolved resistance mechanisms.[151]

The sequencing of full genomes has allowed the monitoring of genome-wide gene expression changes under different circumstances. Such genomics approaches have extended beyond gene expression and gene function analysis in terms of biochemistry or physiology. The rapid developments in molecular techniques allow the analysis of ecological gene functions.[40,82,123] This is the subject of the emerging research field of ecological or environmental genomics. It allows the exploitation of knowledge on mechanisms to develop delicate experiments on the ecological functions of (groups of) genes. This rapidly developing research field is likely to make an enormous contribution to our understanding of the ecology of insect–plant interactions.[40,81]

10.12 Conclusions

The ecology of insect–plant interactions has developed from the ecology of plant–herbivore interactions to the ecology of multitrophic interactions. Recent developments involve the inclusion of interactions with other community members, ranging from microorganisms to mammals, both above and below ground. Furthermore, ecological studies of insect–plant interactions rapidly incorporate mechanistic studies from the subcellular to the individual level, so as to develop manipulative tools to investigate the effects of a species' characteristics on interactions with community members and finally on community processes.

It is becoming increasingly clear that indirect species interactions, non-lethal effects, and on-average small effects can have significant effects on community processes and composition. To understand the ecology of insect–plant interactions, two main forces are explicitly or implicitly considered in many studies, top-down and bottom-up effects, and for a long time the discussion centred on which of these effects was most important. However, these two types of effect usually interact and therefore the question should rather be how the combination of the two impacts on the ecology of insect–plant interactions.

Molecular biology provides ecologists with exciting tools to investigate the ecology of insect–plant interactions. This relates both to the monitoring of changes in individuals in response to exposure to different conditions and to manipulative experiments in which an individual's characteristics are modified to investigate the effects in ecological communities. Future developments in our knowledge of the ecology of insect–plant interactions will benefit enormously from the integration of mechanistic studies with studies of ecological functions.

10.13 References

1. Abrams, P.A., Menge, B.A., and Mittelbach, G.G. (1996). The role of indirect effects in food webs. In *Food webs: integration of patterns and dynamics* (ed. G.A. Polis and K.O. Winemiller), pp. 371–95. Chapman & Hall, New York.
2. Agrawal, A.A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science*, **294**, 321–6.
3. Arimura, G., Ozawa, R., Shimoda, T., Nishioka, T., Boland, W., and Takabayashi, J. (2000). Herbivory-induced volatiles elicit defence genes in lima bean leaves. *Nature*, **406**, 512–15.
4. Baldwin, I.T. (1998). Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proceedings of the National Academy of Sciences of the USA*, **95**, 8113–18.
5. Baldwin, I.T., Halitschke, R., Kessler, A., and Schittko, U. (2001). Merging molecular and ecological approaches in plant–insect interactions. *Current Opinion in Plant Biology*, **4**, 351–8.
6. Ballabeni, P., Wlodarczyk, M., and Rahier, M. (2001). Does enemy-free space for eggs contribute to a leaf beetle's oviposition preference for a nutritionally inferior host plant? *Functional Ecology*, **15**, 318–24.
7. Barbosa, P. (1988). Natural enemies and herbivore–plant interactions: influence of plant allelochemicals and host specificity. In *Novel aspects of insect–plant interactions* (ed. P. Barbosa and D.K. Letourneau), pp. 201–29. John Wiley, New York.
8. Barbosa, P., Krischik, V.A., and Jones, C.G. (1991). *Microbial mediation of plant–herbivore interactions*. John Wiley, New York.
9. Belovsky, G.E. and Slade, J.B. (2000). Insect herbivory accelerates nutrient cycling and increases plant production. *Proceedings of the National Academy of Sciences of the USA*, **97**, 14412–17.
10. Bentley, B.L. (1977). Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics*, **8**, 407–27.
11. Berenbaum, M.R. (1995). The chemistry of defense: theory and practice. *Proceedings of the National Academy of Sciences of the USA*, **92**, 2–8.
12. Berenbaum, M.R. (2002). Postgenomic chemical ecology: from genetic code to ecological interactions. *Journal of Chemical Ecology*, **28**, 873–96.
13. Berlow, E.L. (1999). Strong effects of weak interactions in ecological communities. *Nature*, **398**, 330–4.
14. Bernays, E. and Graham, M. (1988). On the evolution of host specificity in phytophagous arthropods. *Ecology*, **69**, 886–92.
15. Bernays, E.A. (2001). Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annual Review of Entomology*, **46**, 703–27.
16. Bezemer, T.M., Wagenaar, R., Van Dam, N.M., and Wäckers, F.L. (2003). Interactions between above- and below-ground insect herbivores as mediated by the plant defense system. *Oikos*, **101**, 555–62.
17. Bezemer, T.M., Wagenaar, R., Van Dam, N.M., Van Der Putten, W.H., and Wäckers, F.L. (2004). Above- and below-ground terpenoid aldehyde induction in cotton, *Gossypium herbaceum*, following root and leaf injury. *Journal of Chemical Ecology*, **30**, 53–67.

18. Bjørnson, S. and Schütte, C. (2003). Pathogens of mass-produced natural enemies and pollinators. In *Quality control and production of biological control agents—theory and testing procedures* (ed. J.C. Van Lenteren), pp. 133–65. CABI, Oxon.
19. Bonsall, M.B. and Hassell, M.P. (1997). Apparent competition structures ecological assemblages. *Nature*, **388**, 371–3.
20. Breeuwer, J.A.J. and Jacobs, G. (1996). *Wolbachia*: intracellular manipulators of mite reproduction. *Experimental and Applied Acarology*, **20**, 421–34.
21. Brew, C.R., O'Dowd, D.J., and Rae, I.D. (1989). Seed dispersal by ants: behaviour-releasing compounds in elaiosomes. *Oecologia*, **80**, 490–7.
22. Chaneton, E.J. and Bonsall, M.B. (2000). Enemy-mediated apparent competition: empirical patterns and the evidence. *Oikos*, **88**, 380–94.
23. Chen, D.Q., Montllor, C.B., and Purcell, A.H. (2000). Fitness effects of two facultative endosymbiotic bacteria on the pea aphid, *Acyrthosiphon pisum*, and the blue alfalfa aphid, *A. kondoi*. *Entomologia Experimentalis et Applicata*, **95**, 315–23.
24. Chew, F.S. and Renwick, J.A.A. (1995). Host plant choice in *Pieris* butterflies. In *Chemical ecology of insects* (ed. R.T. Cardé and W.J. Bell), pp. 214–38. Chapman & Hall, New York.
25. Crawley, M.J. (1989). Insect herbivores and plant population dynamics. *Annual Review of Entomology*, **34**, 531–64.
26. Crawley, M.J. (1989). The relative importance of vertebrate and invertebrate herbivores in plant population dynamics. In *Insect–plant interactions*, Vol. 1 (ed. E.A. Bernays), pp. 45–71. CRC Press, Boca Raton.
27. Crawley, M.J. (1997). Plant-herbivore dynamics. In *Plant ecology* (ed. M.J. Crawley), pp. 401–74. Blackwell Science, Oxford.
28. Damman, H. (1993). Patterns of interaction among herbivore species. In *Caterpillars. Ecological and evolutionary constraints on foraging* (ed. N.E. Stamp and T.M. Casey), pp. 132–69. Chapman & Hall, New York.
29. Darby, A.C., Birkle, L.M., Turner, S.L., and Douglas, A.E. (2001). An aphid-borne bacterium allied to the secondary symbionts of whitefly. *FEMS Microbiology Ecology*, **36**, 43–50.
30. De Deyn, G.B., Raaijmakers, C.E., Zoomer, H.R., Berg, M.P., De Ruiter, P.C., Verhoef, H.A., *et al.* (2003). Soil invertebrate fauna enhances grassland succession and diversity. *Nature*, **422**, 711–13.
31. De Jong, P.W., De Vos, H., and Nielsen, J.K. (2001). Demic structure and its relation with the distribution of an adaptive trait in Danish flea beetles. *Molecular Ecology*, **10**, 1323–32.
32. Deleted.
33. Denno, R.F., McClure, M.S., and Ott, J.R. (1995). Interspecific interaction in phytophagous insects: competition reexamined and resurrected. *Annual Review of Entomology*, **40**, 297–331.
34. Dicke, M. (1996). The role of microorganisms in tritrophic interactions in systems consisting of plants, herbivores, and carnivores. In *Microbial diversity in time and space* (ed. R.R. Colwell, U. Simidu, and K. Ohwada), pp. 71–84. Plenum, New York.
35. Dicke, M. and Vet, L.E.M. (1999). Plant–carnivore interactions: evolutionary and ecological consequences for plant, herbivore and carnivore. In *Herbivores: between plants and predators* (ed. H. Olff, V.K. Brown, and R.H. Drent), pp. 483–520. Blackwell Science, Oxford.
36. Dicke, M. and Van Loon, J.J.A. (2000). Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata*, **97**, 237–49.
37. Dicke, M. and Bruin, J. (2001). Chemical information transfer between plants: back to the future. *Biochemical Systematics and Ecology*, **29**, 981–94.
38. Dicke, M. and Grostal, P. (2001). Chemical detection of natural enemies by arthropods: an ecological perspective. *Annual Review of Ecology and Systematics*, **32**, 1–23.
39. Dicke, M. and Van Poecke, R.P.M. (2002). Signalling in plant–insect interactions: signal transduction in direct and indirect plant defence. In *Plant signal transduction* (ed. D. Scheel and C. Wasternack), pp. 289–316. Oxford University Press, Oxford.
40. Dicke, M., Van Loon, J.J.A., and De Jong, P.W. (2004). Ecogenomics benefits community ecology. *Science*, **305**, 618–19.
41. Dillon, R.J. (2000). Re-assessment of the role of the insect gut microbiota. *Abstracts Book XXIst International Congress of Entomology*, **1**, 51–5.
42. Douglas, A.E. (1998). Nutritional interactions in insect–microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annual Review of Entomology*, **43**, 17–37.
43. Dukas, R. (2001). Effects of perceived danger on flower choice by bees. *Ecology Letters*, **4**, 327–33.
44. Ehrlich, P.R., Murphy, D.D., Singer, M.C., Sherwood, C.B., White, R.R., and Brown, I.L. (1980). Extinction, reduction, stability and increase; the responses of checkerspot butterfly (*Euphydryas*) populations to the California drought. *Oecologia*, **46**, 101–5.

45. Feeny, P. (1970). Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology*, **51**, 565–81.
46. Feeny, P. (1976). Plant apparency and chemical defense. *Recent Advances in Phytochemistry*, **10**, 1–40.
47. Felton, G.W. and Eichenseer, H. (1999). Herbivore saliva and its effect on plant defense against herbivores and pathogens. In *Induced plant defenses against pathogens and herbivores. Biochemistry, ecology and agriculture* (ed. A.A. Agrawal, S. Tuzun, and E. Bent), pp. 19–36. APS Press, St Paul.
48. Gange, A.C., Bower, E., and Brown, V.K. (2002). Differential effects of insect herbivory on arbuscular mycorrhizal colonization. *Oecologia*, **131**, 103–12.
49. Gardner, S.M., Hartley, S.E., Davies, A., and Palmer, S.C.F. (1997). Carabid communities on heather moorlands in Northeast Scotland: the consequences of grazing pressure for communituy diversity. *Biological Conservation*, **81**, 175–286.
50. Gaston, K.J. (1994). *Rarity*. Chapman & Hall, London.
51. Geervliet, J.B.F., Vet, L.E.M., and Dicke, M. (1994). Volatiles from damaged plants as major cues in long-range host-searching by the specialist parasitoid *Cotesia rubecula*. *Entomologia Experimentalis et Applicata*, **73**, 289–97.
52. Geervliet, J.B.F., Vreugdenhil, A.I., Vet, L.E.M., and Dicke, M. (1998). Learning to discriminate between infochemicals from different plant–host complexes by the parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae). *Entomologia Experimentalis et Applicata*, **86**, 241–52.
53. Gillespie, R.G. and Roderick, G.K. (2002). Arthropods on islands: colonization, speciation, and conservation. *Annual Review of Entomology*, **47**, 595–632.
54. Gomez, J.M. and Gonzalez-Megias, A. (2002). Asymmetrical interactions between ungulates and phytophagous insects: being different matters. *Ecology*, **83**, 203–11.
55. Gouinguene, S., Degen, T., and Turlings, T.C.J. (2001). Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology*, **11**, 9–16.
56. Gould, F. (1979). Rapid host range evolution in a population of the phytophagous mite *Tetranychus urticae* Koch. *Evolution*, **33**, 791–802.
57. Haggstrom, H. and Larsson, S. (1995). Slow larval growth on a suboptimal willow results in high predation mortality in the leaf beetle *Galerucella lineola*. *Oecologia*, **104**, 308–15.
58. Hairston, N.G., Smith, F.E., and Slobodkin, L.B. (1960). Community structure, population control, and competition. *American Naturalist*, **94**, 421–5.
59. Halitschke, R., Gase, K., Hui, D.Q., Schmidt, D.D., and Baldwin, I.T. (2003). Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. VI. Microarray analysis reveals that most herbivore-specific transcriptional changes are mediated by fatty acid-amino acid conjugates. *Plant Physiology*, **131**, 1894–902.
60. Halitschke, R., Ziegler, J., Keinanen, M., and Baldwin, I.T. (2004). Silencing of hydroperoxide lyase and allene oxide synthase reveals substrate and defense signaling crosstalk in *Nicotiana attenuata*. *Plant Journal*, **40**, 35–46.
61. Hamback, P.A. (2001). Direct and indirect effects of herbivory: feeding by spittlebugs affects pollinator visitation rates and seedset of *Rudbeckia hirta*. *Ecoscience*, **8**, 45–50.
62. Hamm, J.J., Styer, E.L., and Lewis, W.J. (1988). A baculovirus pathogenic to the parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae). *Journal of Invertebrate Pathology*, **52**, 189–91.
63. Harmon, J.P., Ives, A.R., Losey, J.E., Olson, A.C., and Rauwald, K.S. (2000). *Coleomegilla maculata* (Coleoptera: Coccinellidae) predation on pea aphids promoted by proximity to dandelions. *Oecologia*, **125**, 543–8.
64. Haukioja, E. and Hakala, T. (1975). Herbivore cycles and periodic outbreaks. Formulation of a general hypothesis. *Reports from the Kevo Subarctic Research Station*, **12**, 1–9.
65. Heidel, A.J. and Baldwin, I.T. (2004). Microarray analysis of salicylic acid- and jasmonic acid-signalling in responses of *Nicotiana attenuata* to attack by insects from multiple feeding guilds. *Plant Cell and Environment*, **27**, 1362–73.
66. Heil, M., Koch, T., Hilpert, A., Fiala, B., Boland, W., and Linsenmair, K.E. (2001). Extrafloral nectar production of the ant-associated plant, *Macaranga tanarius*, is an induced, indirect, defensive response elicited by jasmonic acid. *Proceedings of the National Academy of Sciences of the USA*, **98**, 1083–8.
67. Heil, M. (2004). Induction of two indirect defences benefits Lima bean (*Phaseolus lunatus*, Fabaceae) in nature. *Journal of Ecology*, **92**, 527–36.
68. Herms, D.A. and Mattson, W.J. (1992). The dilemma of plants: to grow or to defend. *Quarterly Review of Biology*, **67**, 283–335.
69. Hermsmeier, D., Schittko, U., and Baldwin, I.T. (2001). Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. I. Large-scale changes in the accumulation of growth- and

defense-related plant mRNAs. *Plant Physiology*, **125**, 683–700.

70. Hilker, M. and Meiners, T. (2002). Induction of plant responses to oviposition and feeding by herbivorous arthropods: a comparison. *Entomologia Experimentalis et Applicata*, **104**, 181–92.
71. Höller, C., Micha, S.G., Schulz, S., Francke, W., and Pickett, J.A. (1994). Enemy-induced dispersal in a parasitic wasp. *Experientia*, **50**, 182–5.
72. Holt, R.D. and Lawton, J.H. (1994). The ecological consequences of shared natural enemies. *Annual Review of Ecology and Systematics*, **25**, 495–520.
73. Holt, R.D. (2000). Trophic cascades in terrestrial ecosystems. Reflections on Polis *et al. Trends in Ecology and Evolution*, **15**, 444–5.
74. Hunter, M.D. and Price, P.W. (1992). Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology*, **73**, 724–32.
75. Jermy, T. (1988). Can predation lead to narrow food specialization in phytophagous insects? *Ecology*, **69**, 902–4.
76. Jones, C.G. (1984). Microorganisms as mediators of plant resource exploitation by insect herbivores. In *A new ecology. Novel approaches to interactive systems* (ed. P.W. Price, C.N. Slobodchikoff, and W.S. Gaud), pp. 53–99. Wiley, New York.
77. Jones, C.G., Ostfeld, R.S., Richard, M.P., Schauber, E.M., and Wolff, J.O. (1998). Chain reactions linking acorns to gypsy moth outbreaks and Lyme disease risk. *Science*, **279**, 1023–6.
78. Kahl, J., Siemens, D.H., Aerts, R.J., Gäbler, R., Kühnemann, F., Preston, C.A., *et al*. (2000). Herbivore-induced ethylene suppresses a direct defense but not a putative indirect defense against an adapted herbivore. *Planta*, **210**, 336–42.
79. Karban, R., Agrawal, A.A., and Mangel, M. (1997). The benefits of induced defenses against herbivores. *Ecology*, **78**, 1351–5.
80. Kareiva, P. and Sahakian, R. (1990). Tritrophic effects of a simple architectural mutation in pea plants. *Nature*, **345**, 433–4.
81. Kessler, A. and Baldwin, I.T. (2002). Plant responses to insect herbivory: the emerging molecular analysis. *Annual Review of Plant Biology*, **53**, 299–328.
82. Kessler, A., Halitschke, R., and Baldwin, I.T. (2004). Silencing the jasmonate cascade: induced plant defenses and insect populations. *Science*, **305**, 665–8.
83. Koptur, S. (1992). Extrafloral nectary-mediated interactions between insects and plants. In *Insect–plant interactions*, Vol. 4 (ed. E.A. Bernays), pp. 81–129. CRC Press, Boca Raton.
84. Krebs, J.R. and Kacelnik, A. (1991). Decision making. In *Behavioural ecology: an evolutionary approach* (ed. J.R. Krebs and N.B. Davies), pp. 105–36. Blackwell Scientific, Oxford.
85. Krischik, V.A., Barbosa, P., and Reichelderfer, C.F. (1988). Three trophic level interactions: allelochemicals, *Manduca sexta* (L.), and *Bacillus thuringiensis* var. *kurstaki* Berliner. *Environmental Entomology*, **17**, 476–82.
86. Kruess, A. and Tscharntke, T. (2000). Species richness and parasitism in a fragmented landscape: experiments and field studies with insects on *Vicia sepium. Oecologia*, **122**, 129–37.
87. Landolt, P.J. (1993). Effects of host plant leaf damage on cabbage looper moth attraction and oviposition. *Entomologia Experimentalis et Applicata*, **67**, 79–85.
88. Lawton, J.H. and Schröder, D. (1977). Effect of plant type, size of geographical range and taxonomic isolation on number of insect species associated with British plants. *Nature*, **265**, 137–40.
89. Lawton, J.H., Lewinsohn, T.M., and Compton, S.G. (1993). Patterns of diversity for insect herbivores on bracken. In *Species diversity in ecological communities* (ed. R.E. Ricklefs and D. Schluter), pp. 178–84. University of Chicago Press, Chicago.
90. Lou, Y.G. and Baldwin, I.T. (2004). Nitrogen supply influences herbivore-induced direct and indirect defenses and transcriptional responses to *Nicotiana attenuata. Plant Physiology*, **135**, 496–506.
91. Louda, S.M. and Potvin, M.A. (1995). Effect of inflorescence feeding insects on the demography and lifetime fitness of a native plant. *Ecology*, **76**, 229–45.
92. Louda, S.M. and Rodman, J.E. (1996). Insect herbivory as a major factor in the shade distribution of a native crucifer (*Cardamine cordifolia* A. Gray, bittercress). *Journal of Ecology*, **84**, 229–37.
93. Luck, R.F. and Podoler, H. (1985). Competitive exclusion of *Aphytis lingnanensis* by *A. melinus*: potential role of host size. *Ecology*, **66**, 904–13.
94. Mantyla, E., Klemola, T., and Haukioja, E. (2004). Attraction of willow warblers to sawfly-damaged mountain birches: novel function of inducible plant defences? *Ecology Letters*, **7**, 915–18.
95. Maron, J.L. and Gardner, S.N. (2000). Consumer pressure, seed versus safe-site limitation, and plant population dynamics. *Oecologia*, **124**, 260–9.
96. Marquis, R.J. and Whelan, C.J. (1994). Insectivorous birds increase growth of white oak through consumption of leaf-chewing insects. *Ecology*, **75**, 2007–14.
97. Mayhew, P.J. (2001). Herbivore host choice and optimal bad motherhood. *Trends in Ecology and Evolution*, **16**, 165–7.

98. McCann, K.S., Hastings, A., and Huxel, G.R. (1998). Weak trophic interactions and the balance of nature. *Nature*, **395**, 794–8.
99. McConn, M., Creelman, R.A., Bell, E., Mullet, J.E., and Browse, J. (1997). Jasmonate is essential for insect defense in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the USA*, **94**, 5473–7.
100. McIntosh, A.R. and Townsend, C.R. (1996). Interactions between fish, grazing invertebrates and algae in a New Zealand stream: a trophic cascade mediated by fish induced changes to grazer behaviour? *Oecologia*, **108**, 174–81.
101. Memmott, J. (1999). The structure of a plant–pollinator food web. *Ecology Letters*, **2**, 276–80.
102. Memmott, J., Fowler, S.V., Paynter, Q., Sheppard, A.W., and Syrett, P. (2000). The invertebrate fauna on broom, *Cytisus scoparius*, in two native and two exotic habitats. *Acta Oecologca*, **21**, 213–22.
103. Mitchell-Olds, T. (2001). *Arabidopsis thaliana* and its wild relatives: a model system for ecology and evolution. *Trends in Ecology and Evolution*, **16**, 693–700.
104. Moran, N.A. and Whitham, T.G. (1990). Interspecific competition between root-feeding and leaf-galling aphids mediated by host-plant resistance. *Ecology*, **7**, 1050–8.
105. Muller, C.B. and Godfray, H.C.J. (1997). Apparent competition between two aphid species. *Journal of Animal Ecology*, **66**, 57–64.
106. Muller, C.B., Adriaanse, I.C.T., Belshaw, R., and Godfray, H.C.J. (1999). The structure of an aphid–parasitoid community. *Journal of Animal Ecology*, **68**, 346–70.
107. Musser, R.O., Hum-Musser, S.M., Eichenseer, H., Peiffer, M., Ervin, G., Murphy, J.B., *et al.* (2002). Caterpillar saliva beats plant defences—a new weapon emerges in the evolutionary arms race between plants and herbivores. *Nature*, **416**, 599–600.
108. Ohsaki, N. and Sato, Y. (1994). Food plant choice of *Pieris* butterflies as a trade-off between parasitoid avoidance and quality of plants. *Ecology*, **75**, 59–68.
109. Omacini, M., Chaneton, E.J., Ghersa, C.M., and Muller, C.B. (2001). Symbiotic fungal endophytes control insect host–parasite interaction webs. *Nature*, **409**, 78–81.
110. Pace, M.L., Cole, J.J, Carpenter, S.R., and Kitchell, J.F. (1999). Trophic cascades revealed in diverse ecosystems. *Trends in Ecology and Evolution*, **14**, 483–8.
111. Papaj, D.R. (1986). Shifts in foraging behavior by a *Battus philenor* population: field evidence for switching by individual butterflies. *Behavioral Ecology and Sociobiology*, **19**, 31–9.
112. Paul, N.D., Hatcher, P.E., and Taylor, J.E. (2000). Coping with multiple enemies: an integration of molecular and ecological perspectives. *Trends in Plant Science*, **5**, 220–5.
113. Pieterse, C.M.J. and Van Loon, L.C. (1999). Salicylic acid-independent plant defence pathways. *Trends in Plant Science*, **4**, 52–8.
114. Polis, G.A. (1999). Why are parts of the world green? Multiple factors control productivity and the distribution of biomass. *Oikos*, **86**, 3–15.
115. Polis, G.A., Sears, A.L.W., Huxel, G.R., Strong, D.R., and Maron, J. (2000). When is a trophic cascade a trophic cascade? *Trends in Ecology and Evolution*, **15**, 473–5.
116. Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N., and Weis, A.E. (1980). Interactions among three trophic levels: influence of plant on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, **11**, 41–65.
117. Price, P.W. (1997). *Insect ecology*. Academic Press, New York.
118. Prieur-Richard, A.H., Lavorel, S., Linhart, Y.B., and Dos Santos, A. (2002). Plant diversity, herbivory and resistance of a plant community to invasion in Mediterranean annual communities. *Oecologia*, **130**, 96–104.
119. Prokopy, R.J. and Roitberg, B.D. (2001). Joining and avoidance behavior in nonsocial insects. *Annual Review of Entomology*, **46**, 631–65.
120. Rambo, J.L. and Faeth, S.H. (1999). Effect of vertebrate grazing on plant and insect community structure. *Conservtion Biology*, **13**, 1047–54.
121. Raymond, B., Vanbergen, A., Pearce, I., Hartley, S.E., Cory, J.S., and Hails, R.S. (2002). Host plant species can influence the fitness of herbivore pathogens: the winter moth and its nucleopolyhedrovirus. *Oecologia*, **131**, 533–41.
122. Reitz, S.R. and Trumble, J.T. (2002). Competitive displacement among insects and arachnids. *Annual Review of Entomology*, **47**, 435–65.
123. Reymond, P., Weber, H., Damond, M., and Farmer, E.E. (2000). Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis. Plant Cell*, **12**, 707–19.
124. Reymond, P., Bodenhausen, N., Van Poecke, R.M.P., Krishnamurthy, V., Dicke, M., and Farmer, E.E. (2004). A conserved transcript pattern in response to a specialist and a generalist herbivore. *Plant Cell*, **16**, 3132–47.
125. Ritchie, M.E. and Olff, H. (1999) Herbivore diversity and plant dynamics: compensatory and additive

effects. In *Herbivores: between plants and predators* (ed. H. Olff, V.K. Brown, and R.H. Drent), pp. 175–204. Blackwell Science, Oxford.

126. Roitberg, B.D., Robertson, I.C., and Tyerman, J.G.A. (1999). Vive la variance: a functional oviposition theory for insect herbivores. *Entomologia Experimentalis et Applicata*, **91**, 187–94.
127. Root, R.B. (1973). Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards *(Brassica oleracea). Ecological Monographs*, **43**, 95–124.
128. Root, R.B. and Cappuccino, N. (1992). Patterns in population change and the organization of the insect community associated with goldenrod. *Ecological Monographs*, **62**, 393–420.
129. Rostas, M. and Hilker, M. (2003). Indirect interactions between a phytopathogenic and an entomopathogenic fungus. *Naturwissenschaften*, **90**, 63–7.
130. Rott, A.S. and Godfray, H.C.J. (2000). The structure of a leafminer–parasitoid community. *Journal of Animal Ecology*, **69**, 274–89.
131. Ruhren, S. and Handel, S.N. (1999). Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. *Oecologia*, **119**, 227–30.
132. Schmitz, O.J., Hamback, P.A., and Beckerman, A.P. (2000). Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. *American Naturalist*, **155**, 141–53.
133. Schoonhoven, L.M. (1990). Host-marking pheromones in Lepidoptera, with special reference to two *Pieris* spp. *Journal of Chemical Ecology*, **16**, 3043–52.
134. Schultz, J.C. (1988). Many factors influence the evolution of herbivore diets, but plant chemistry is central. *Ecology*, **69**, 896–7.
135. Schütte, C., Van Baarlen, P., Dijkman, H., and Dicke, M. (1998). Change in foraging behaviour of the predatory mite *Phytoseiulus persimilis* after exposure to dead conspecifics and their products. *Entomologia Experimentalis et Applicata*, **88**, 295–300.
136. Shiojiri, K., Takabayashi, J., Yano, S., and Takafuji, A. (2001). Infochemically mediated tritrophic interaction webs on cabbage plants. *Population Ecology*, **43**, 23–9.
137. Shiojiri, K., Takabayashi, J., Yano, S., and Takafuji, A. (2002). Oviposition preferences of herbivores are affected by tritrophic interaction webs. *Ecology Letters*, **5**, 186–92.
138. Simberloff, D.S. and Wilson, E.O. (1969). Experimental zoogeography of islands: the colonization of empty islands. *Ecology*, **50**, 278–96.
139. Snyder, W.E. and Wise, D.H. (2000). Antipredator behavior of spotted cucumber beetles (Coleoptera: Chrysomelidae) in response to predators that pose varying risks. *Environmental Entomology*, **29**, 35–42.
140. Southwood, T.R.E., Moran, V.C., and Kennedy, C.E.J. (1982). The richness, abundance and biomass of the arthropod communities on trees. *Journal of Animal Ecology*, **51**, 635–6.
141. Speight, M.R., Hunter, M.D., and Watt, A.D. (1999). *Ecology of insects. Concepts and applications*. Blackwell Science, Oxford.
142. Stanforth, L.M., Louda, S.M., and Bevill, R.L. (1997). Insect herbivory on juveniles of a threatened plant, *Cirsium pitcheri*, in relation to plant size, density and distribution. *Ecoscience*, **4**, 57–66.
143. Stapley, L. (1998). The interaction of thorns and symbiotic ants as an effective defence mechanism of swollen-thorn acacias. *Oecologia*, **115**, 401–5.
144. Steffan-Dewenter, I. and Tscharntke, T. (1999). Effects of habitat isolation on pollinator communities and seed set. *Oecologia*, **121**, 432–40.
145. Stephens, D.W. and Krebs, J.R. (1986). *Foraging theory*. Princeton University Press, Princeton.
146. Stotz, H.U., Pittgendrigh, B.R., Kroymann, J., Weniger, K., Fritsche, J., Bauke, A., *et al*. (2000). Induced plant defense responses against chewing insects. Ethylene signaling reduces resistance of *Arabidopsis* against Egyptian cotton worm but not diamondback moth. *Plant Physiology*, **124**, 1007–17.
147. Stouthamer, R., Breeuwer, J.A.J., and Hurst, G.D.D. (1999). *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annual Review of Microbiology*, **53**, 71–102.
148. Strauss, S.Y. (1997). Floral characters link herbivores, pollinators, and plant fitness. *Ecology*, **78**, 1640–5.
149. Strong, D.R., Lawton, J.H., and Southwood, T.R.E. (1984). *Insects on plants: community patterns and mechanisms*. Harvard University Press, Cambridge, MA.
150. Strong, D.R. (1992). Are trophic cascades all wet? Differentiation and donor-control in speciose ecosystems. *Ecology*, **73**, 747–54.
151. Tattersall, D.B., Bak, S., Jones, P.R., Olsen, C.E., Nielsen, J.K., Hansen, M.L., *et al*. (2001). Resistance to an herbivore through engineered cyanogenic glucoside synthesis. *Science*, **293**, 1826–8.
152. Thaler, J.S., Fidantsef, A.L., Duffey, S.S., and Bostock, R.M. (1999). Trade-offs in plant defense against pathogens and herbivores: a field demonstration of chemical elicitors of induced resistance. *Journal of Chemical Ecology*, **25**, 1597–609.
153. Thaler, J.S. (2002). Effect of jasmonate-induced plant responses on the natural enemies of herbivores. *Journal of Animal Ecology*, **71**, 141–1.

154. Thaler, J.S., Stout, M.J., Karban, R., and Duffey, S.S. (2001). Jasmonate-mediated induced plant resistance affects a community of herbivores. *Ecological Entomology*, **26**, 312–24.
155. Thibout, E., Guillot, J.F., Ferary, S., Limouzin, P., and Auger, J. (1995). Origin and identification of bacteria which produce kairomones in the frass of *Acrolepiopsis assectella* (Lep, Hyponomeutoidea). *Experientia*, **51**, 1073–5.
156. Thies, C. and Tscharntke, T. (1999). Landscape structure and biological control in agroecosystems. *Science*, **285**, 893–5.
157. Thomas, C.D. (1986). Butterfly larvae reduce host plant survival in vicinity of alternative host species. *Oecologia*, **70**, 113–17.
158. Thomas, J. and Lewington, R. (1991). *The butterflies of Britain and Ireland*. Dorling Kindersly, London.
159. Tindall, K.V. and Stout, M.J. (2001). Plant-mediated interactions between the rice water weevil and fall armyworm in rice. *Entomologia Experimentalis et Applicata*, **101**, 9–17.
160. Trumble, J.T., Kolodny-Hirsch, D.M., and Ting, I.P. (1993). Plant compensation for arthropod herbivory. *Annual Review of Entomology*, **38**, 93–119.
161. Tscharntke, T., Vidal, S., and Hawkins, B.A. (2001). Parasitoids of grass-feeding chalcid wasps: a comparison of German and British communities. *Oecologia*, **129**, 445–51.
162. Turlings, T.C.J., Wäckers, F.L., Vet, L.E.M., Lewis, W.J., and Tumlinson, J.H. (1993). Learning of host-finding cues by hymenopterous parasitoids. In *Insect learning: ecological and evolutionary perspectives* (ed. D.R. Papaj and A.C. Lewis), pp. 51–78. Chapman & Hall, New York.
163. Turlings, T.C.J., Loughrin, J.H., McCall, P.J., Rose, U.S.R., Lewis, W.J., and Tumlinson, J.H. (1995). How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences of the USA*, **92**, 4169–74.
164. Underwood, N. (1999). The influence of plant and herbivore characteristics on the interaction between induced resistance and herbivore population dynamics. *American Naturalist*, **153**, 282–94.
165. Van Dam, N.M. and Baldwin, I.T. (1998). Costs of jasmonate-induced responses in plants competing for limited resources. *Ecology Letters*, **1**, 30–3.
166. Van der Meijden, E., Wijn, M., and Verkaar, H.J. (1988). Defence and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos*, **51**, 355–63.
167. Van der Putten, W.H., Vet, L.E.M., Harvey, J.H., and Wäckers, F.L. (2001). Linking above- and below-ground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology and Evolution*, **16**, 547–54.
168. Van Loon, J.J.A., De Boer, J.G., and Dicke, M. (2000). Parasitoid–plant mutualism: parasitoid attack of herbivore increases plant reproduction. *Entomologia Experimentalis et Applicata*, **97**, 219–27.
169. Van Loon, J.J.A. and Dicke, M. (2000). Sensory ecology of arthropods utilizing plant infochemicals. In *Sensory ecology* (ed. F.G. Barth and A. Schmid), pp. 253–70. Springer, Heidelberg.
170. Van Poecke, R.M.P., Posthumus, M.A., and Dicke, M. (2001). Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: chemical, behavioral, and gene-expression analysis. *Journal of Chemical Ecology*, **27**, 1911–28.
171. Van Poecke, R.M.P. and Dicke, M. (2004). Indirect defence of plants against herbivores: using *Arabidopsis thaliana* as a model plant. *Plant Biology*, **6**, 387–401.
172. Van Rijn, P.C.J., Van Houten, Y.M., and Sabelis, M.W. (2002). How plants benefit from providing food to predators even when it is also edible to herbivores. *Ecology*, **83**, 2664–79.
173. Van Tol, R.W.H.M., Van der Sommen, A.T.C., Boff, M.I.C., Van Bezooijen, J., Sabelis, M.W., and Smits, P.H. (2001). Plants protect their roots by alerting the enemies of grubs. *Ecology Letters*, **4**, 292–4.
174. Vet, L.E.M., Wäckers, F.L., and Dicke, M. (1991). How to hunt for hiding hosts: the reliability–detectability problem in foraging parasitoids. *Netherlands Journal of Zoology*, **41**, 202–13.
175. Vet, L.E.M. and Dicke, M. (1992). Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, **37**, 141–72.
176. Vet, L.E.M., Lewis, W.J., and Cardé, R.T. (1995). Parasitoid foraging and learning. In *Chemical ecology of insects* (ed. R.T. Cardé and W.J. Bell), pp. 65–101. Chapman & Hall, New York.
177. Vet, L.E.M. (1996). Parasitoid foraging: the importance of variation in individual behaviour for population dynamics. In *Frontiers of population ecology* (ed. R.B. Floyd and A.W. Sheppard), pp. 245–56. CSIRO Publishing, Melbourne.
178. Visser, J.H. (1986). Host odor perception in phytophagous insects. *Annual Review of Entomology*, **31**, 121–44.
179. Visser, M.E. and Holleman, L.J.M. (2001). Warmer springs disrupt the synchrony of oak and winter moth phenology. *Proceedings of the Royal Society of London, Series B, Biological Sciences*, **268**, 289–94.

180. Voelckel, C. and Baldwin, I.T. (2004). Generalist and specialist lepidopteran larvae elicit different transcriptional responses in *Nicotiana attenuata*, which correlate with larval FAC profiles. *Ecology Letters*, **7**, 770–5.

181. Vos, M., Moreno Berrocal, S., Karamaouna, F., Hemerik, L., and Vet, L.E.M. (2001). Plant-mediated indirect effects and the persistence of parasitoid–herbivore communities. *Ecology Letters*, **4**, 38–45.

182. Vrieling, K., Smit, W., and Van der Meijden, E. (1991). Tritrophic interactions between aphids (*Aphis jacobaeae* Schrank), ant species, *Tyria jacobaeae* L. and *Senecio jacobaea* L. lead to maintenance of genetic variation in pyrrolizidine alkaloid concentration. *Oecologia*, **86**, 177–82.

183. Wäckers, F.L. and Bezemer, T.M. (2003). Root herbivory induces an above-ground indirect defence. *Ecology Letters*, **6**, 9–12.

184. Wäckers, F.L., Zuber, D., Wunderlin, R., and Keller, F. (2001). The effect of herbivory on temporal and spatial dynamics of foliar nectar production in cotton and castor. *Annals of Botany*, **87**, 365–70.

185. Walker, M. and Jones, T.H. (2001). Relative roles of top-down and bottom-up forces in terrestrial tritrophic plant–insect herbivore–natural enemy systems. *Oikos*, **93**, 177–87.

186. Walling, L.L. (2000). The myriad plant responses to herbivores. *Journal of Plant Growth Regulation*, **19**, 195–216.

187. Williams, I.S., Jones, T.H., and Hartley, S.E. (2001) The role of resources and natural enemies in determining the distribution of an insect herbivore population. *Ecological Entomology*, **26**, 204–11.

188. Wootton, J.T. (1994). The nature and consequences of indirect effects in ecological communities. *Annual Review of Ecology and Systematics*, **25**, 443–66.

189. Wratten, S. (1992). Population regulation in insect herbivores—top-down or bottom-up? *New Zealand Journal of Ecology*, **16**, 145–7.

190. Yano, S. (1994). Flower nectar of an autogamous perennial *Rorippa indica* as an indirect defense mechanism against herbivorous insects. *Researches on Population Ecology*, **36**, 63–71.

191. Zamora, R. and Gomez, J.M. (1993). Vertebrate herbivores as predators of insect herbivores: an asymmetrical interaction mediated by size differences. *Oikos*, **6**, 223–8.

CHAPTER 11

Evolution: insects and plants forever in combat

Insects have an amazing capability to adapt to changing environmental conditions. For instance, since the introduction of chemical pesticides in the 1940s insects have rapidly evolved resistance against a large number of these compounds,[32] to an extent where industry is now facing ever more serious problems in finding new insecticidal compounds (Fig. 11.1). This has been described as providing a modern metaphor and circumstantial evidence for insect herbivore counteradaptation to novel secondary plant metabolites.[27] The astonishing adaptive capability of herbivorous insects is also clear from the speed at which pest insects such as the Hessian fly or the brown plant-hopper can adapt into biotypes that are virulent on newly bred resistant crop varieties.[103] Analogously, it cannot escape attention that plants can undergo drastic evolutionary changes under artificial selection by, for example, plant breeders. Fossil records have shown the evolution of new species of plants and insects over hundreds of millions of years. As a consequence, it will be no surprise that insect–plant interactions are constantly being modified by natural selection. However, the mechanisms that underlie the evolution of interactions between plants and insects are not easily uncovered and are the topic of an ongoing debate. In 1888 E. Stahl wrote: 'Thus, the animal world which surrounds the plants deeply influenced not only their morphology, but also their chemistry'[52] and in 1964

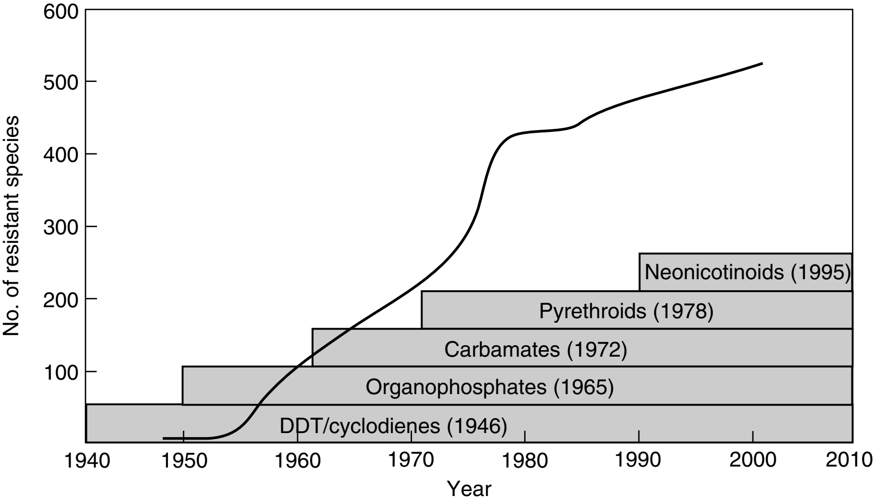

Figure 11.1 Synthetic insecticides have been produced since 1939, the year when DDT was developed. The first case of DDT resistance was recorded in 1946. Over the next 60 years, the number of resistant arthropod species (line) increased rapidly in response to the development and use of insecticides. Bars indicate period when particular insecticide groups have been used, and dates in parentheses are the year in which resistance was first documented. (After Denholm *et al.*, 2002.[32] Reproduced with permission. © 2002 American Association for the Advancement of Science.)

Ehrlich and Raven put forward their theory on the co-evolution of insects and plants.[43] The resulting discussion was intense and ample new investigations have been initiated so that our concept of how insect–plant interactions evolve is evolving as well. In this chapter we present the state of the art of this ongoing debate that deals with specialization, speciation, and evolution of insect–plant interactions.

11.1 Fossilized records of insect–plant interactions

The face of planet Earth has changed drastically over the past aeons (Fig. 11.2). A large variety of gymnosperm species dominated plant biodiversity until the early Cretaceous, about 144 million years ago. The oldest insect and plant fossils date from approximately 400 million years ago. Both taxa have diversified throughout the Mesozoic period (roughly 400–140 million years ago). Insect herbivores have evolved various ways of exploiting living plants as food. Among insect-related fossils, a plethora of examples can be found of feeding damage caused by arthropods (Fig. 11.3).[82] Fossil records indicate that the earliest arthropod herbivory occurred in the early Devonian (ca. 400 million years ago) in the form of spore feeding and the piercing–sucking feeding habit.[80] External feeding and galling are reported from the middle and late Pennsylvanian (310–290 million years ago), respectively. External foliage feeding in the form of hole feeding and skeletonization is known from the early Permian (ca. 290 million years ago).[80] The sequence in which the different feeding modes most likely evolved is from sucking to chewing, whereas mining and galling were established later.[121]

The first plants are somewhat older than the first insects, but the currently largest group of plants (the angiosperms) arose in the Cretaceous period when insects were abundantly present.[81] The time relationship between the diversification of the main herbivorous insect groups and the dramatic diversification of the angiosperms towards the end of the Early Cretaceous has been the subject of much discussion, because it may help to determine whether plants enhanced the evolution of the insects or whether the opposite interaction was also important. The extensive spread of several extant

Figure 11.2 Reconstructed view of Late Carboniferous vegetation. Pteridophytic trees reached their fullest expression in the warm swamps of this period. Tree ferns of great height, rising to over 50 m, grew with an understory of bushy and herbaceous ferns and horsetails. Seed plants, such as the conifer *Cordaites* (upper right corner), grew to about 30 m with a trunk diameter of 1 m. This plant had long, strap-like leaves up to 1 m in length and 15 cm across. (From Mägdefrau, 1959, with permission.)[87]

herbivorous insect orders, especially Lepidoptera, Coleoptera (Chrysomelidae, Curculionidae), Diptera (Agromyzidae, Cecidomyidae), and Hymenoptera (Cynipidae), occurred after the appearance and radiation of angiosperms.[121] This would suggest that the appearance and evolution of the flowering plants accelerated the evolution of these groups. Other palaeontological data show, however, that the familial radiation (increase in the number of families within the orders) in several modern insects began 245 million years ago, about 100 million years earlier than the appearance and rise to dominance of the angiosperms, which occurred 144–66 million years ago.[81] This might support the hypothesis that insect diversity has influenced angiosperm diversity. The spindle diagrams of Figure 11.4 indicate that the familial radiation of Coleoptera, Lepidoptera, Diptera, and Hymenoptera occurred in the Upper Mesozoic, that is, coinciding with the appearance and ascendancy of the flowering plants which, thus, may have accelerated the radiation of these groups of insects. No such effect, however, is apparent in Orthoptera, Homoptera, and Heteroptera. Analysing the number of all insect families through time, Labandeira and Sepkoski even came to the conclusion that the appearance and ascendancy of the angiosperms coincided with a slowdown rather than an acceleration of insect familial diversification (Fig. 11.5).[81] We have to emphasize, however, that familial diversification is not necessarily identical to species diversification. Thus, from the presently available palaeontological information, there seems to be no general coincidence in time between the evolution

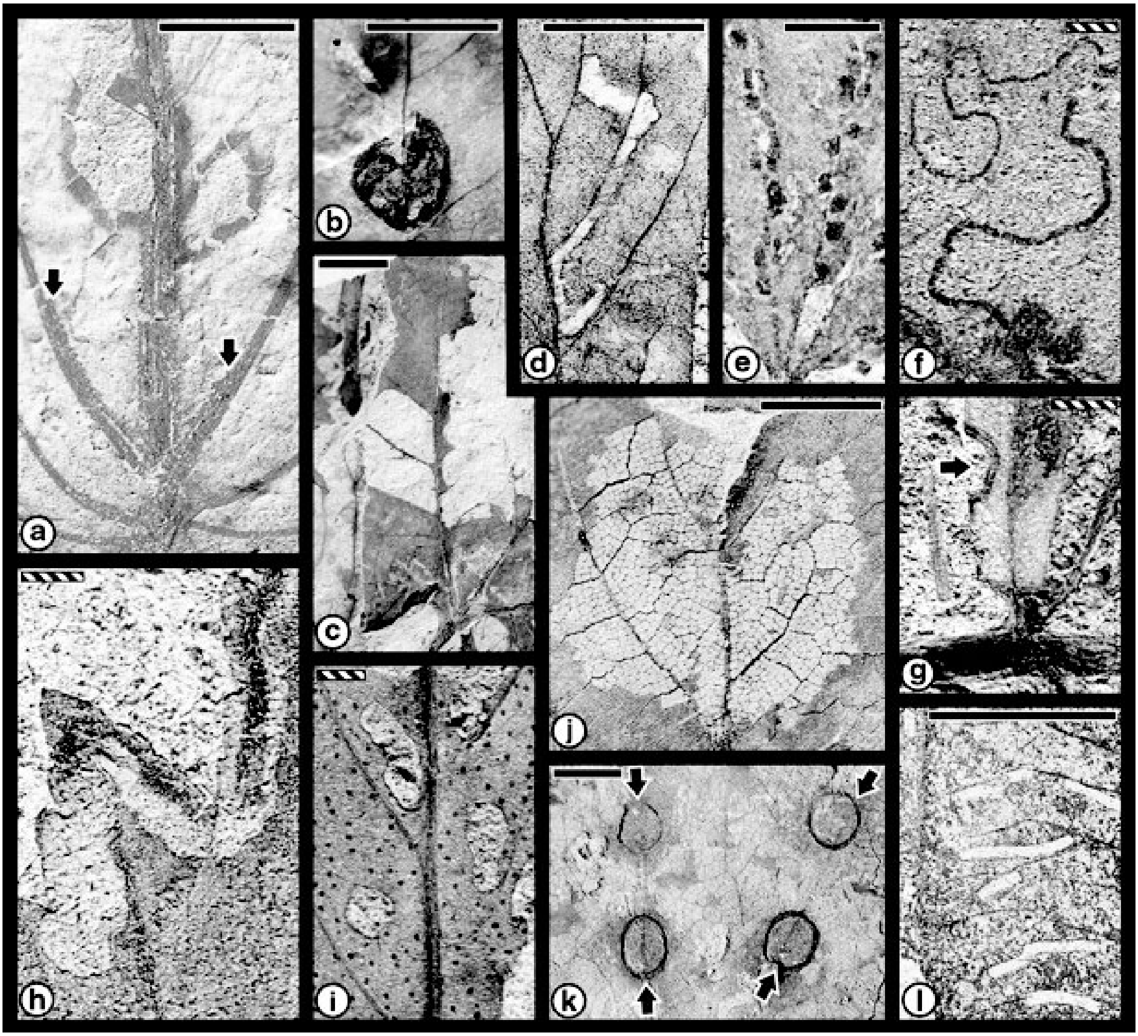

Figure 11.3 A spectrum of plant–insect associations from the Williston Basin of southwestern North Dakota. Associations range from the earliest Paleocene at upper left and continue to the older associations of the latest Cretaceous at lower right. All material is from the Denver Museum of Nature and Science or the Yale Peabody Museum. Scale bars: solid = 1 cm, backslashed = 0.1 cm. (**a**) Two linear mines with oviposition sites (arrows), following secondary and then primary venation, terminating in a large pupation chamber on the dicot *Paranymphaea crassifolia*. (**b**) Single gall on primary vein of *Cercidiphyllum genetrix* (Cercidiphyllaceae). (**c**) Free feeding damage on *Platanus raynoldsi* (Platanaceae). (**d**) Skeletonization on a probable lauralean leaf. (e) Multiple galls on *Trochodendroides nebrascensis* (Cercidiphyllaceae). (**f**) Initial phase of a serpentine mine on *Marmarthia pearsonii* (Lauraceae). (**g**) Cuspate margin feeding (arrow) on *Metasequoia* sp. (Cupressaceae). (**h**) Serpentine leaf-mine assigned to the Nepticulidae (Lepidoptera), on unidentified Rosaceae. (**i**) Hole feeding pattern on an unknown genus of Urticales. (**j**) General skeletonization on *Erlingdorfia montana* (Platanaceae). (**k**) Large scale-insect impressions centred on primary veins of *E. montana*. (**l**) Slot hole feeding on an unidentified genus of Platanaceae. (From Labandeira *et al*., 2002.)[82]

of higher plants and insect taxa. This may, however, be due to the high level of integration of these observations. The family level may not be the right level for such analyses. This is supported by an investigation of interactions between chrysomelid beetles of the genus Blepharida and their Burseraceae host plants. This study showed that the plant defences and the beetle counterdefences evolved in synchrony over the last 112 million years, during most of the era of the angiosperms.[7]

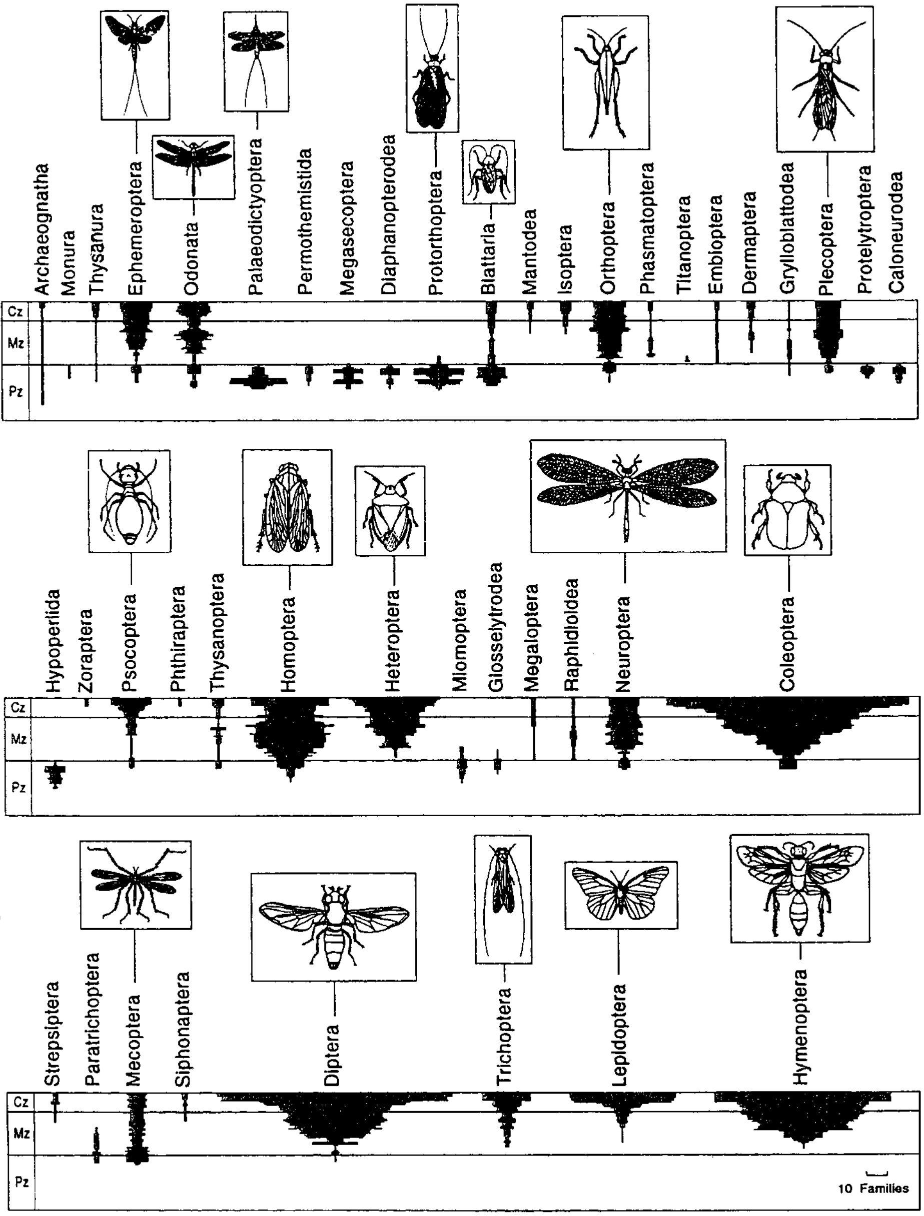

Figure 11.4 Spindle diagrams displaying diversities in fossil families with insect orders in stratigraphic stages of the Phanerozoic. A scale bar is shown in the lower right corner. Pz, Paleozoic; Mz, Mesozoic; Cz, Czenozoic. Angiosperms appeared approximately two-thirds of the way up the band for Mesozoic (i.e. above the 'M' in 'Mz'). (From Labandeira and Sepkoski, 1993.[81] © 1993 American Association for the Advancement of Science, with permission.)

11.2 Speciation

Insects are the most speciose group of multicellular organisms and therefore constitute the major component of biodiversity. The origin of biological species (i.e. species that are reproductively isolated) has been discussed intensively[94] since Darwin published his landmark book 'On the origin of

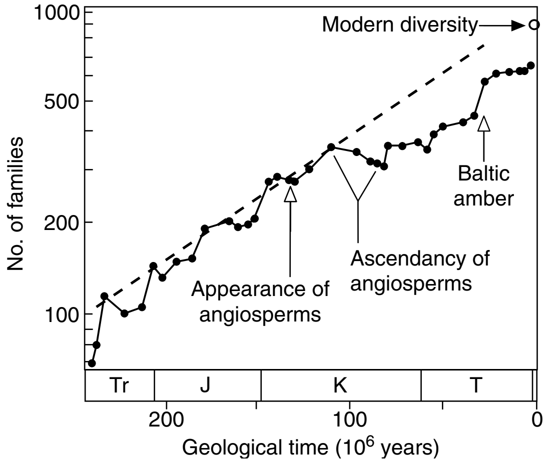

Figure 11.5 Insect familial diversity from the Triassic to the recent, plotted on semilogarithmic coordinates. The dashed line is interpretative, illustrating possible exponential diversification beginning in the Triassic and possibly continuing into the Early Cretaceous. Tr, Triassic; J, Jurassic; K, Cretaceous; T, Tertiary. (From Labandeira and Sepkoski, 1993.[81] © 1993 American Association for the Advancement of Science, with permission.)

species'.[30] Likewise the origin of insect species and their specialization has been discussed intensively in the twentieth century, especially with respect to reciprocal evolution in insect–plant interactions.[43,74,125] The history of the theory of the origin of species in general, and of insects in particular, has been excellently reviewed by Schilthuizen.[113]

Most herbivorous insect species are specialists, and even species that are considered generalists may be composed of populations of specialists.[51] Speciation is classically assumed to occur allopatrically, for example in populations that are geographically isolated and as a result cannot exchange genes. However, after a long scientific debate it has become clear that speciation can also occur sympatrically[11] and herbivorous insects have made a first well documented case, as shown in the research by Bush and colleagues on the apple maggot fly *Rhagoletis pomonella*.[18] The apple maggot fly originally developed in hawthorn fruits but apple was later incorporated in the diet as well. Flies that preferred to oviposit in apple also mated on apple plants, whereas flies that preferred to oviposit on hawthorn mated with other flies on hawthorn, a phenomenon called assortative mating. In addition, the life histories were characterized by allochrony (see Section 11.2.1c). As a result, a preference for hawthorn in a subpopulation of the original population resulted in reproductive isolation, which favoured sympatric speciation. Many other examples for herbivorous insects followed,[11] such as tree-hoppers,[109] chrysomelid beetles,[104] aphids,[21] and small ermine moths.[95] As a result, the concept of sympatric speciation is now well accepted. Sympatric speciation occurs when a new species develops within the parental species' area of distribution, that is, within the 'cruising range' of individuals of the parental species. In pure allopatry, gene flow between populations is hindered at the very beginning by an extrinsic barrier, whereas in pure sympatry gene flow may continue to some extent for a number of generations after the populations have become separated.

Whereas allopatric speciation operates on a larger geographical scale, sympatric speciation results in a mosaic of speciation in which new species evolve at very close distances from the original population. This also has consequences for how we view the dynamics and spatial scale of biodiversity.

11.2.1 Reproductive isolation

The emergence of a daughter species from a parental species is possible only if some barrier prevents or restricts the gene flow between two populations. Below we discuss some types of barrier that were found in herbivorous insects. They cause either premating reproductive isolation (spatial and behavioural barriers, allochrony in life history) or postmating isolation (hybrid incompatibility).

(a) Spatial barriers

The most obvious barrier to gene flow is the geographical isolation of populations by a mountain range, sea, river, desert, etc. The efficiency of such barriers depends largely on the dispersal capacity of the insects. For species with a sedentary lifestyle even relatively short distances may be sufficient for effective isolation. This is indicated by the genetic differences found between such populations. For example, the chrysomelid species *Oreina cacaliae* and *O. globosa*, which are oligophagous on *Petasites*, *Senecio*, and *Adenostyles* species (Asteraceae), show considerable genetic divergence, as estimated by electrophoretic methods, among populations separated by only 40–250 km in Switzerland and Germany.[40] Surprisingly, even distances of 10 m to a few hundred metres may suffice for spatial isolation. The distribution of resistance genes (R genes) among populations of the flea beetle *Phyllotreta nemorum* in Denmark also showed a strong population structure. These genes provide the beetles with the ability to feed on a specific strain of the crucifer *Barbarea vulgaris*, whereas beetles lacking these R genes die when feeding on this plant.[31] Non-dispersive monophagous insects such as scale insects, leaf-miners and gall-midges living on trees were found to represent genetically highly different subpopulations (termed 'demes') on individual trees. Insects transferred from one tree to another tree of the same species performed poorly compared with conspecific insects transferred within the same tree. This is explainable by the fact that such insects may live for hundreds of generations on the same tree. Thus, the phytochemical and microhabitat differences among individual trees, acting as selective forces, may result in genetically different demes.[98]

(b) Behavioural barriers

Differences in feeding and/or oviposition preferences of herbivorous insects can result in effective isolation and, therefore, most probably offer an opportunity for sympatric speciation. In such cases gene flow can be totally absent between coexisting insect populations narrowly specialized to different host-plant species. For example, apple maggot (*Rhagoletis pomonella*) adults are attracted to specific chemicals that occur in apples, whereas a closely related (sibling) species, *R. mendax*, is attracted by different chemicals to the blueberry fruit.[53] Although the two species can easily be hybridized in the laboratory, genetic analysis has shown that under natural conditions there is no gene flow between them, because mating occurs only on their respective host plants.[20,47,48] Likewise, the aphid *Aulacorthum solani* s. str., which is polyphagous but avoids *Pulmonaria officinalis*, does not hybridize in nature with *A. solani langei*, which lives monophagously on *P. officinalis*. The two subspecies are totally separated by their different host-plant preferences, even though their host plants grow intermingled and often in physical contact with each other. Another subspecies, however, *A. s. aegopodii*, which feeds monophagously on *Aegopodium podagraria*, may occasionally hybridize with *A. solani* s. str. (Fig. 11.6).[100]

Other types of behavioural barrier between populations are caused by, for example, differences in the composition of sex pheromones between two European corn borer (*Ostrinia nubilalis*) populations in North America[79] and France,[105] or by differences in acoustic mate recognition signals in plant-hoppers (Homoptera).[26] In the case of the European corn borer in France, it appears that different host-plant races have different sex pheromone compositions and that no cross-attraction to the sex pheromone from another race occurs.[105] This resembles the situation with the apple maggot fly where host-plant preference is linked to reproductive isolation.

Not only insect-produced infochemicals, but also herbivore-induced plant cues, can mediate reproductive isolation. Larvae of the cynipid gall wasp *Antistrophus rufus* feed within galls inside the flowering stems of the asteraceous prairie perennials *Silphium laciniatum* and *Silphium terebinthinaceum*. Allozyme studies revealed that subpopulations in the two plant species were reproductively isolated and that terpenoid plant volatiles induced by the feeding wasps mediated this through an effect on mate localization by adult males.[129]

(c) Allochrony in life history

Allochrony (the opposite of synchrony) in various parts of insects' life histories, especially in mating periods, may cause reproductive isolation. For instance, the North American membracid species complex, *Enchenopa binotata*, contains nine sympatric species specialized on coexisting host trees. Their allochronic life histories on different hosts are the primary factor in initiating and maintaining reproductive isolation. This has led to asynchronic mating periods and ultimately to speciation.[141] As shown in Figure 11.7, the emergence pattern of three apple maggot races overlaps only partially, thereby probably strongly reducing mating among the races.[19] The question arises, however, whether allochrony was the cause or the result of speciation.

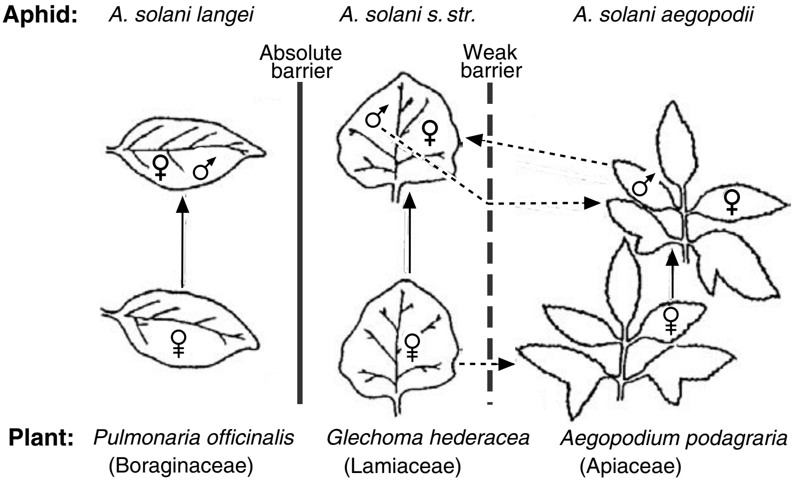

Figure 11.6 Premating isolating barriers for *Aulacorthum* aphids, with different strengths caused by differentially strong preferences for host plants. (From Müller, 1985.)[100]

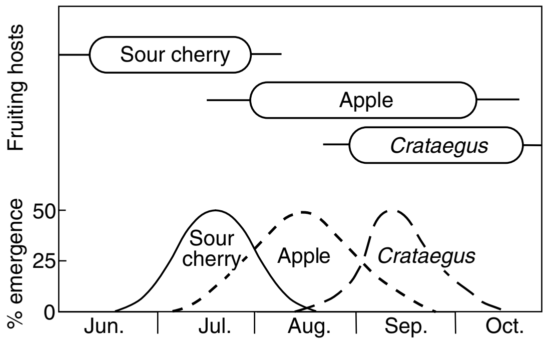

Figure 11.7 Adult emergence pattern of three host races of *Rhagoletis pomonella* in Wisconsin and fruiting time of the hosts. The last switch from *Crataegus* to apple occurred after apple was introduced into the USA 150 years ago. (From Bush, 1975.)[19]

(d) Hybrid incompatibility

Different populations of the same species may differ genetically to such an extent that the zygotes of hybrids are non-viable. For example, European populations of the cherry fruit fly *Rhagoletis cerasi* are divided into at least two geographical races (Fig. 11.8), which show unidirectional incompatibility: crosses between males of the southern complex (circles) and females of the north-east populations (triangles) produce low hatch rates of eggs, whereas the reciprocal crosses yield normal levels of fertility. This unilateral incompatibility may be due to either genetic or cytoplasmic factors. A third cause may be related to the absence or presence of microbial symbionts.[17] According to Thompson,[123] symbionts in concert with environmental factors often play an important role in speciation. The interaction between a given symbiont and its host may be antagonistic in one environment but commensal or even mutualistic in other environments. In this way, differential selection exerted by different environments on the symbiont–host interactions may magnify the differences among different insect populations and thereby lead to speciation. An example of symbiont-caused incompatibility is found in the alfalfa weevil *Hypera postica*. In experiments with three American and one European population, the crosses between populations harbouring a *Rickettsia* and populations free of *Rickettsia* proved incompatibility.[66] The widespread symbiont *Wolbachia*, which can have a wide range of effects on its hosts including cytoplasmic incompatibility, has often been assumed to play a role in insect speciation. A recent analysis of the available data, however, led to the view that it is too early to draw this conclusion and that the role of nuclear genes and other symbionts, either alone or in concert with *Wolbachia*, in insect speciation may have been overlooked.[137] For instance, in the cricket species complex *Allonemobius fasciatus-socius*, molecular data indicate that three species diverged from a common ancestor in the presence

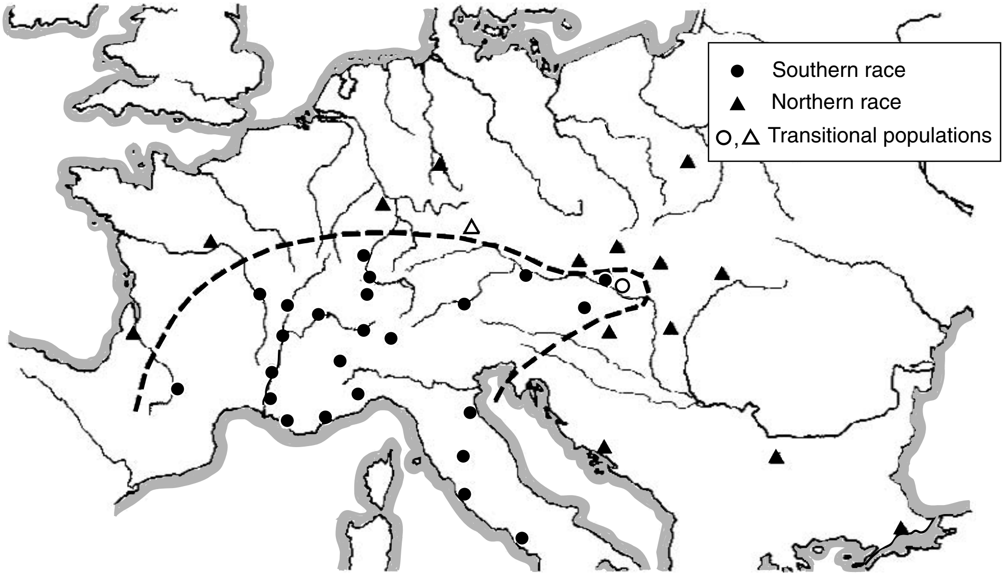

Figure 11.8 Distribution of the southern and northern races of *Rhagoletis cerasi* which show incompatibility. (From Boller *et al.*, 1976.)[17]

of one strain of *Wolbachia*, thus suggesting a minimal role for *Wolbachia* during this burst of speciation.[90]

11.2.2 Rates of speciation

The number of new (daughter) species emerging per unit of time is determined primarily by the occurrence of genetic variation through time in the parental species and secondarily by the forces of natural selection and drift. Evidence for a primary role of genetic variation is provided by studies on young oceanic islands such as the Big Island of the Hawaiian Archipelago, which began to emerge from the Pacific Ocean less than 400 000 years ago. As mentioned above, here the rate of specialization in various species following colonization by an ancestral immigrant varied greatly. This can be explained by differences in the propensity of genomes for genetic disorganization and reorganization (speciose versus non-speciose lineages).[22] Why some gene complexes are so stable while others evolve rapidly (genetic revolution) remains an as yet unsolved problem.[93]

The rate of speciation depends also on generation span, because the majority of mutations arise during meiosis. Herbivorous insects show great variability in this respect. For example, the development of the North American periodical cicada *Magicicada septendecim* lasts for 17 years,[139] whereas the diamondback moth *Plutella xylostella* has up to 28 generations per year in tropical environments.[64] Clearly, assuming the same mutation rate, species with short generation times—such as most insects—are potentially more prone to fast speciation than those with long generation times, such as perennial plants. Thus, a herbivorous insect species' adaptation to the evolutionary changes of its host tree is potentially much faster than the evolutionary response of the tree to attack by the insect, supposing equally strong selection pressures exerted by the partners on one another.

11.2.3 Reciprocal speciation

Reciprocal speciation as a consequence of interactions between organisms is called diversifying co-evolution.[124] Ehrlich and Raven implicitly supposed that the interaction between herbivorous insects and plants might result in speciation of both partners.[43] In an extensive review of the vast literature on co-evolution, Thompson demonstrated how different ecological, genetic, and phylogenetic conditions influence specialization and the co-evolutionary process.[125] We consider this intensively debated issue in Section 11.8.

11.3 Genetic variation in host-plant preference of insects

For evolution to occur, there should be genetic variation that results in differential fitness. Variability in a population of organisms has a combined phenotypic and genotypic basis. Modern biometric genetics attempts to partition the phenotypic variability into components derived, respectively, from variability in the genes and variability in the environment. The basis for evolutionary changes is formed by the combination of genetic variation and natural selection. In this section we focus on genetic variation in host preferences between closely related insect species, populations, and individuals. Studies that have attempted to unravel the genetic background of host-plant preference are relatively few and often incomplete, but their results are highly rewarding.

11.3.1 Interspecific differences

Interspecific variation was studied in some closely related *Yponomeuta* species. The F_1 progeny of crosses between *Y. cagnagellus*, specialized on *Euonymus europaeus* (Celastraceae), and *Y. malinellus*, specialized on apple (Rosaceae), accepted the host plants of both parents in spite of the taxonomic distance between the two foods. There is some evidence that in the F_1 progeny of the interspecific hybrid chemoreceptor sensitivity to characteristic host chemicals of both parents is autosomally combined.[131] Frey *et al.* demonstrated clear-cut differences between the electroantennograms of the apple and the hawthorn races of *Rhagoletis pomonella* and the closely related *R. mendax* to several host fruit odour components (Fig. 11.9).[55] The differences were heritable. Thus, antennal sensitivity to volatile plant substances presumably plays a role in host shifts and speciation in these insects. Hybrids between the two species show a significantly weaker antennal response to volatile compounds compared with that of the hosts of either parent. This presumably reflects a reduced ability to locate host plants, and this may be the cause of absence of gene flow in nature.[54]

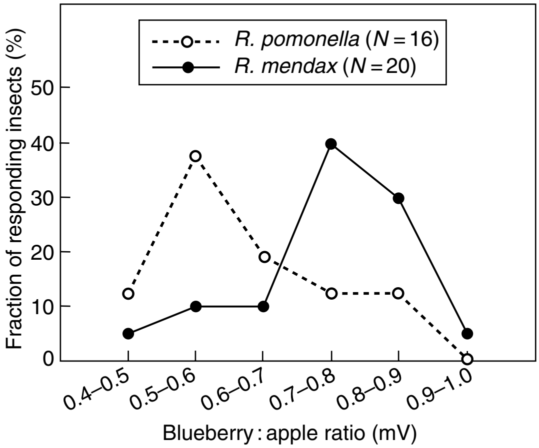

Figure 11.9 Frequency distribution of electroantennogram (EAG) responses of two sibling fruit fly species, *Rhagoletis pomonella* and *R. mendax*, expressed as the ratio between EAG amplitudes when stimulated by pentane extracts of apple and blueberry, the respective host plants of both species. Overlap of response patterns is caused by some individuals showing responses that are typical for their sibling species. (From Frey and Bush, 1990.)[53]

Females of a swallowtail butterfly, *Papilio zeliacon*, population from the western part of North America oviposit on two umbellifer species, *Lomatium grayi* and *Cymopterus terebinthus*, whereas females of the closely related *P. oregonius* population, at a distance of about 50 km from the above population, oviposit exclusively on *Artemisia dracunculus* (Asteraceae), although both other plant species are also available. Laboratory crosses between the two species have shown that oviposition preference in these species is controlled significantly by one or more loci on the X chromosome and is modified by at least one locus on other chromosomes.[124] In several other hybridization experiments host preferences and performance traits have often been found to be polygenically based, either autosomally or (partially) sex linked (Table 11.1). This finding may be relevant for the observation that hybrids often demonstrate an expanded host range relative to either parent.

11.3.2 Intraspecific differences

Heritable intraspecific variation in host-plant preferences has been found in several insect species in

Table 11.1 Host-plant preferences and larval performance of interspecific hybrids and intraspecific crosses

Species	Food-plant preference	Ovipositional preference	Performance	A or X	Remarks	Reference
Interspecific hybrids						
Helicoverpa v. × s.	Dominance			A		5
Helicoverpa v. × s.			Dominance	A	Performance is determined polygenically	117
Yponomeuta c. × m.	Both parents			A	Information on chemoreceptor sensitivity in hybrids	131
Lymenitis a. × a.	Both parents				Information on preference induction in hybrids	63
Papilio o. × z.			Intermediate	A	Genes affecting performance differ from those affecting oviposition preference	128
Papilio o. × z.		P. dominance		X(A)	X chromosome has the largest effect, modifying effects by autosomal gene(s)	122
Papilio	Dominance				One parent species is monophagous, the other is polyphagous	120
Papilio g. × c.				A	Genes affecting performance differ from those affecting oviposition preference	116
Papilio g. × c.				X	Oviposition site preference probably based on relatively few loci	116
Procecidochares a. × A		Dominance		A	Oviposition site preference based on single-gene, two-allele system	67
Intraspecific crosses (different populations or strains)						
Oncopeltus fasciatus		Dominance			Oviposition site preference is inherited polygenically	84
Drosophila tripunctata		Dominance		A	Oviposition site preference is inherited polygenically	69
Drosophila tripunctata	P. dominance			A	Food preference is genetically independent of oviposition site preference	68
Helicoverpa virescens		Dominance		X	Oviposition site preference is inherited with paternal dominance	135

A, autosomal genes involved; X, sex-linked genes involved; P, partial (in contrast to most other animals, Lepidoptera (and birds) have a sex determination system in which females are the heterogametic sex).

laboratory assays and in plant breeding for insect resistance. For example, females of laboratory-reared isofemale strains of the oligophagous swallowtail *Papilio zeliacon* and the monophagous *P. oregonius* showed intraspecific variation among strains in the propensity to lay some eggs on the other species' host plants.[124] Females of *Helicoverpa virescens* from Mississippi show a greater oviposition preference for cotton than those collected at the US Virgin Islands. Crosses have indicated that this difference is genetically determined and most probably resulted from a difference in the abundance of cotton between the two locations.[114] Some strains of the silkworm *Bombyx mori* that readily accept several plants other than mulberry to feed on possess deterrent receptors with a strikingly reduced sensitivity to some secondary plant compounds that in normal silkworms strongly inhibit feeding.[4] *Uroleucon ambrosiae* aphids show geographical variation in host-plant preference. In eastern North America the aphids specialize on giant ragweed (*Ambrosia trifida*), whereas a variety of other asteraceous plants are used as host in the southwest of the USA.[58]

Oviposition specificity of the polyphagous nymphalid butterfly *Polygonia c-album* is strongly sex linked, as appeared from a study that made reciprocal crosses of two populations that differ in the degree of specialization. The genes mediating oviposition specificity are therefore located primarily on the X chromosome, which a female butterfly inherits from her father.[70]

Thus, a population of herbivorous insects consists of a collection of different genotypes that display a range of host-plant preference phenotypes. Depending on local circumstances, such as the relative abundance of host-plant species, some genotypes are likely to be favoured over others by natural selection.

11.3.3 Preference–performance correlation

Studies on the genetic co-variance between oviposition preference and larval performance have produced conflicting results.[127,133] The reason that often no co-variance can be demonstrated may be that many studies were based on average measures for populations, whereas the evolution of co-variance acts at the level of heritable differences among individuals. Alternatively, the selection pressure on adults may be different from that on juveniles. For instance, adults may be under selection to optimize foraging success, which does not necessarily coincide with selecting the best host plants for their offspring.[111] In the few cases where the presence of preference–performance co-variance was sought at the level of individuals, a significant co-variance could be detected,[23,101,112] although not for the flea beetle *Phyllotreta nemorum*.[102]

A lack of genetic variation for either host preference behaviour or digestive capability may hinder the evolution of correspondence.[136] Such incongruity is often quite evident in cases of introduced plants. North American *Pieris* species, for instance, readily oviposit on the crucifer *Thlaspi arvense*, although it is toxic to their larvae. This plant was introduced at the end of the nineteenth century, and either there has not been enough time to evolve discrimination against it in the butterfly[23] or, alternatively, a shortage of adequate genetic variation in host-choice behaviour prevented such an evolutionary change in the insect's behaviour.

11.3.4 Genetic variation and local host-plant adaptation

Since the 1990s there has been intense interest in local processes and the role of population structure in the evolution of insect–plant interactions.[98,125] Local adaptation in structured populations, such as meta-populations, is likely to result in locally adaptive gene complexes that are tightly linked. If patches with different host-plant genotypes provide a herbivore population with different opportunities, genetically different herbivore subpopulations may result because local selection favours particular genotypes that are best adapted to the local host-plant genotypes. Different genes may confer adaptation and these may become genetically linked and result in co-adapted gene complexes. These co-adapted gene complexes break down during hybridization with individuals of other subpopulations, resulting in outbreeding depression. As a result hybrids have a lower fitness, and thus selection favours the offspring of local mating.[31] The structured occurrence of populations is an important concept in our understanding of the role of intraspecific genetic variation in adaptive evolution. Flea beetles (*Phyllotreta nemorum*) collected from the G-type genotype of *Barbarea vulgaris* spp. *arcuata* are all homozygous for an R gene that confers resistance in the beetles to the plant's defence. Beetle populations sampled on other host plants had much lower frequencies of beetles with R genes

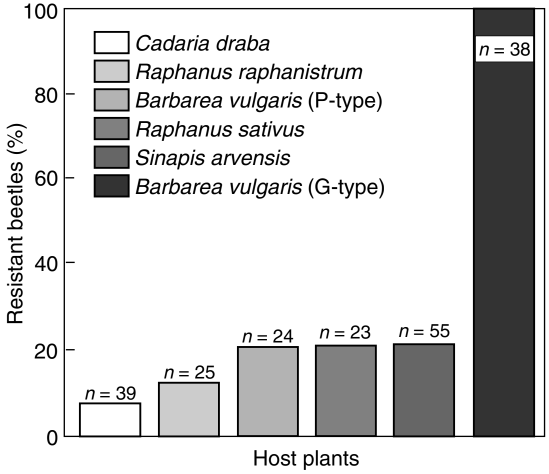

Figure 11.10 Proportion of resistant *Phyllotreta nemorum* that were collected on different host plants. Samples collected from geographically distinct populations of the same plant species were pooled. (Modified from De Jong and Nielsen, 2002,[31] with kind permission of Springer Science and Business Media.)

(Fig. 11.10). However, some of these populations were only a few kilometres away from the populations on the G-type *Barbarea* plants, and the beetles can easily cover such distances. Genetic studies have shown that homozygous resistant lines (RR) obtained in the laboratory through back-crossing have a strong reduction in larval survival on the G-type *Barbarea* plants compared with homozygous resistant beetles collected in the field. The likely explanation for this is the existence of a gene complex that includes modifiers that offset the negative pleiotropic effects of the homozygous resistance gene. These homozygous resistant backcrossed lines may have lost the linkage of modifiers to the R genes and therefore suffer from pleiotropic effects.[31]

Molecular tools will provide a major step forward for unravelling genetic mechanisms in co-adapted gene complexes. Such investigations should address population structure and selective regimes that influence migration, gene flow, and adaptation.[39,99] It is important to note that molecular genetic tools, originally available only for model organisms such as *Drosophila*, are becoming rapidly available for non-model species as well. Therefore, the years to come are likely to show major progress in this area.

11.4 Genetic variation in plant resistance against insects

Plants can be attacked by a multitude of mobile enemies and have many options for defence against herbivorous insects (see Chapters 3, 4, and 10). The different modes of defence may negatively influence one another. Therefore, one should not be surprised to find polymorphisms with respect to defences against herbivorous insects. For instance, ragwort (*Senecio jacobaea*) populations are polymorphic for concentrations of pyrrolizidine alkaloids (PAs). High concentrations of these secondary metabolites support the plant's direct defence, which is effective against several herbivore species, including aphids, but not against the specialist herbivore *Tyria jacobaeae*. In contrast, low concentrations support the plant's indirect defence against *T. jacobaeae* via ants that visit the plant to collect aphid honeydew. The ants also prey on the caterpillars of *T. jacobaeae*. Therefore, plant individuals with high PA concentrations thrive best in years with few *T. jacobaeae*, and plants with low PA concentrations do best in years with many *T. jacobaeae*.[134] Variation in selective pressure from the specialist herbivore and from generalist herbivores can explain the maintenance of genetic variation in the plant population. Apart from ecological costs, such as in the case of defences in *Senecio jacobaeae*, there are also direct costs in terms of fitness loss associated with plant defences.[6,65]

It is fairly common to note differences in damage among individual plants within a population. These differences may reflect differences in environmental characteristics such as light and previous damage, or they may reflect genetic differences. Additionally, environmental differences may also be the result of a genetic factor. For instance, a genetic difference can influence the amount of previous damage that has induced resistance. Genetic variation for resistance against insects has been documented in a large number of plant species, including agricultural and natural species. However, information on the genetic mechanisms underlying resistance is more abundant for agricultural species and shows that many genetic mechanisms mediate resistance against insects.[77] For instance, antibiosis in alfalfa against the pea aphid *Acyrthosiphon pisum* is mediated by one or a few genes, whereas resistance against the spotted alfalfa aphid *Therioaphis maculata* has a polygenic background. Moreover, in muskmelon (*Cucumis melo*), antibiosis against the melon aphid *Aphis gossypii* is mainly monogenic, and additional genes have minor effects.[77] A comparison of wild tobacco (*Nicotiana attenuata*) populations from two different states in the USA showed differences in the degree of constitutive and inducible direct defence through trypsin protease inhibitors, as well as inducible indirect defence through herbivore-induced plant volatiles, that correlated with the degree of herbivore damage. The increased levels of trypsin protease inhibitors came with a fitness cost to the plants.[61] In wild parsnip plants (*Pastinaca sativa*), attack by the parsnip webworm *Depressaria pastinacella* varies within populations, sometimes resulting in a total loss of lifetime fitness (i.e. total seed production). Genetic studies have shown that the number of primary umbel seeds lost to herbivory is heritable and genetically correlated to heritable variation in furanocoumarin content.[9]

In conclusion, genetic differences among plant individuals in resistance to herbivorous insects have been amply reported. Thus, both a population of plants and a population of herbivorous insects consists of a mixture of genotypes that differ in costs and benefits related to the resistance and counter-resistance, respectively. This genetic variation provides natural selection with the raw material from which those genotypes that are best adapted in terms of fitness make the largest contribution to the next generation.

11.5 Selection and adaptation

There is abundant experimental evidence that herbivorous arthropods can adapt to their host plants.[76,133] For example, lines of the polyphagous spider mite *Tetranychus urticae* rapidly adapted to a novel host plant species. In fewer than 10 generations, lines kept on tomato or broccoli showed greater acceptance and lower mortality rates than mites from a control line kept on lima bean plants.[57] The abundance of plant secondary metabolites provides, amongst other plant defences, herbivorous insects with a significant barrier to overcome (see also Chapters 4 and 5). This has selected for adaptations in herbivorous insects. For instance, glucosinolates or nicotine are toxic to a wide range of unadapted herbivore species, whereas a limited number of specialist species has adapted to these toxins. For instance, caterpillars of the tobacco specialist *Manduca sexta* effectively excrete the nicotine they ingest in a way that precludes intoxication of the insects. However, this adaptation does not come without costs, as the caterpillars thrive better on tobacco plants that do not produce nicotine as a result of the silencing of an essential step in the biosynthesis of nicotine.[119]

Apart from constitutive defences, plants also possess inducible defences. These defences have generally been regarded as mechanisms to reduce the costs of defence. Although reducing the costs of defence may be an important aspect of induced defences, an additional benefit may be that they confront herbivores with a variable phenotype that reduces the possibilities for the herbivore to adapt to the plant's defence.[2,60] For instance, garlic mustard (*Alliaria petiolata*) plants from different sites in a forest varied in levels of defence compounds, whereas these levels were similar when plants from different sites were grown in a glasshouse.[25] Thus, variation in the field does not seem to reflect a genetic variation in these characters. Phenotypic variation in the expressed plant defences could affect herbivores and slow down adaptation by herbivores. Moreover, foraging for food plants, as well as selection of oviposition sites, shapes herbivore preferences for certain plant individuals over others.[111]

In contrast to the abundant evidence for adaptations of insects to their food plants, experimental evidence for the adaptation of plants to herbivorous insects seems to be scarcer, except for floral characters (see Chapter 12). Only a limited number of examples exists. For instance, herbivore pressure determines the distribution of the crucifer *Cardamine cordifolia*, which is found predominantly in the shade of other plant species, and thus the plants are likely to adapt to living under shaded conditions.[86] Insects were found to exert selection on the resistance of morning glory (*Ipomoea purpurea*) to caterpillars, although the resistance traits have not been identified.[118] In *Arabidopsis thaliana* the experimental removal of herbivores and pathogens showed that these organisms select for increased levels of trichomes and glucosinolates. However, as both pathogens and insects were experimentally removed, it remains unclear what the relative contribution to the selection pressure is of each of the organisms excluded.[91] Moreover, it is well known that plant characteristics can be modified through artificial selection by plant breeders[103] or selection by the application of herbicides.[96] It has been argued in the past that insects do not exert a strong selection on plants because insects do not cause a lot of damage. However, there are good examples of insects that may locally destroy all their host plants, such as the cinnabar moth *T. jacobaeae* that can locally eliminate its host plant *Senecio jacobaea*,[134] or hornworms (*Manduca quinquemaculata* and *M. sexta*) that can locally eliminate all their host plants, the wild tobacco *Nicotiana attenuata*.[106] Herbivorous insects can significantly reduce their host-plant's lifetime fitness under natural conditions.[85] Moreover, the relationship between herbivore damage and the effect on plant fitness is not necessarily linear; even low amounts of damage may incur large fitness costs to plants. For instance, low densities of the cynipid gall wasp *Andricus quercus-calicis* caused a reduction in seed production to 2.5–4.2 times fewer acorns than for uninfested *Quercus robur*.[28] Therefore, there are good reasons to assume that insects have a larger selective impact on plants than is often assumed. A recent manipulative field study has provided excellent experimental support for this. This study showed that insect herbivory had a strong influence on plant growth and survival[49] to the extent that insect herbivory influenced tropical forest diversity by contributing to habitat specialization.[89] The effects of insect herbivory were different for plants from nutrient-poor soils and for plants from nutrient-rich soils. Plants from nutrient-poor soils were highly defended through a high tannin : protein ratio, even when they grew on nutrient-rich soils, whereas plants from nutrient-rich soils were less well defended and grew faster.[49] This investigation demonstrates not only that insect herbivory can exert strong selection on plants but also that different factors, such as biotic plus abiotic stresses, should be incorporated when analysing selective forces. Interestingly, this study provides experimental support for a hypothesis put forward by Janzen 30 years earlier, namely that herbivores and plant defences are the most important factors in the evolution of specialization of plants to nutrient-poor soils and not adaptations to low-nutrient conditions.[72]

Finally, even in those cases where insect damage has only minor effects on plant fitness, the main factor of importance in the context of evolution is whether the effects on plant fitness differ among plant individuals and whether this difference has a genetic basis.

11.6 Evolution of insect diversity

The Insecta are by far the most species-rich taxon. Several hypotheses have been propounded to explain the evolution of the striking diversity of the Insecta and in particular of herbivorous insects.

According to the *ecological saturation hypothesis* there has always been a roughly constant number of niches that could be occupied by insect species or higher insect taxa.[92] A new insect taxon could become established only if another was excluded by competition and became extinct.

The proponents of the *expanding resource hypothesis* argue that the resources provided by plants to insects have increased, both in quantity and in the ratio of niches per resource.[138] In this view, an increase in plant structural and architectural diversity opens new possibilities for an increase in insect diversity. This opinion is well supported by the fact that more complex plants harbour more herbivorous species (see Section 10.6). Nevertheless, this hypothesis also implies that the diversity of herbivorous insects, in general, is determined primarily by the Plant Kingdom.

As mentioned in Chapter 2, herbivory characterizes about 45% of all insect species. Phylogenetic analyses have shown that herbivorous taxa are characterized by higher rates of diversification and speciation than closely related non-herbivorous taxa.[125] It has been estimated that herbivory has arisen at least 50 times among existing taxa.[97] In 11 of 13 sister groups of insects, the herbivorous group contained almost twice the number of species compared with the non-herbivorous group.[97] At the end of the Cretaceous period a major extinction of insect herbivores occurred. An analysis of fossils spanning the Cretaceous–Paleocene boundary in southwestern North Dakota has shown that the most specialized associations, which were diverse and abundant before the event, suffered most and did not easily recover, while generalist associations quickly recovered and were again abundant in younger fossils.[82] This example draws attention to several important aspects related to the evolution of insect–plant interactions: the advantages and disadvantages of a specialist lifestyle among herbivorous insects, selection pressures and speciation, and the evolution of herbivorous insects.

11.7 Evolution of host-plant specialization

Recording that specialization is more frequent among herbivorous insects than generalization is easier than explaining it. The specialist lifestyle is often considered to be derived from a generalist lifestyle. However, there are examples that do not support this view.[125] For instance, no directionality was found towards increasing specialization in the butterfly tribe Nymphalini. The ancestor of the clade was probably a specialist on urticaceous plants and polyphagy is probably a derived state.[71] As we have seen in Sections 11.3 and 11.5, there is variation among individual insects in a population with respect to host-plant selection and preference, and adaptation to new host plants can occur. Various factors have been proposed as agents of selection in the evolution of the specialistic feeding habit of herbivorous insects. The main factors are: (1) coping with plant secondary metabolites, (2) avoiding competition, and (3) reducing mortality from natural enemies.

11.7.1 Coping with plant secondary metabolites

Herbivorous insects are exposed to an immense variety of secondary plant metabolites, which comprise highly toxic compounds such as alkaloids, glucosinolates, and furanocoumarins, to mention just a few (see Chapter 4). Herbivorous insects have various mechanisms of avoiding or detoxifying these plant compounds. Insects that are capable of detoxifying one class of plant compound usually cannot detoxify a very different class of secondary metabolites. Specialist insects using the same plant taxon have evolved different detoxification or excretion mechanisms to avoid the impact of the same secondary plant metabolites (see Chapter 5).[107,140] It has often been considered that detoxification or excretion of secondary plant metabolites releases the herbivores from the negative effects of these plant chemicals. However, several recent studies have shown that this is not true.[3,119] For instance, crucifer-specific secondary metabolites were found to reduce the fitness of the crucifer specialist herbivore *Pieris rapae*.[3] Thus, although specialist herbivores are able to feed on plants rich in certain secondary metabolites, this does not imply that the plant toxins do not affect the herbivore's physiology and fitness. However, being able to detoxify plant secondary metabolites or to avoid exposure allows herbivores to feed on plants that are unsuitable to many other herbivore species.

An exciting investigation has shown that a single plant gene can determine whether the plant will be included in the diet of insect herbivores. Wild tobacco plants silenced in the lipoxygenase gene that mediates a basic step in the jasmonate signalling pathway were successfully attacked by *Empoasca* leaf-hoppers, herbivores that did not feed on wild-type control plants. Moreover, the plants were also more vulnerable to adapted herbivores such as the tobacco hornworm.[78] Thus, on wild-type plants that induce large amounts of nicotine and other chemical defences in response to herbivory, adapted herbivores, such as the tobacco hornworm, can feed while potential competitors that are present in the environment cannot.

In many cases deterrents, rather than toxins, may play a role in speciation and specialization. For instance, the major proximal determinant of host specialization in the pea aphid *Acyrthosiphon pisum* is the behavioural acceptance of a plant rather than its toxicity. The behavioural responses to the deterrents mediate host plant selection as well as assortative mating, because the aphids mate on their food plant.[21] For an extensive overview of the role of deterrents in host plant selection and the evolution of chemoreception, see Section 7.9. Therefore, deterrents from their food plants play a role in specialization and speciation in these phloem-feeding herbivores.

11.7.2 Competition

In a landmark paper on the evolution of insect–plant interactions by Ehrlich and Raven, the escape from interspecific competition among herbivorous insects was introduced as a possible factor in host-plant specialization.[43] For a long time, competition has been discarded as an important issue in host-plant specialization, because competition among herbivorous insects was considered to be unimportant. However, recent accumulating evidence demonstrates that this view is not always correct (see Chapter 10).[29,33] Herbivorous insects compete for food in various ways, including competition among individuals (a) during simultaneous attack of different tissues of a food plant,[130] (b) in temporally separated attacks (e.g. through induced plant responses), and (c) in spatially separated attacks, for example by diverting nutrient flows within the plant or by inducing systemic resistance that affects other herbivores at distant parts of the same plant individual. For example, prior feeding by sap-feeding *Prokelisia* plant-hoppers on cordgrass negatively affected plant-hoppers in the next generation. This effect was asymmetrical, as *Prokelisia dolus* had a stronger effect on *P. marginata* than vice versa. This delayed interspecific competition seems to be mediated by changes in the nutritional value of the plant, most likely in amino acid levels.[34]

The consequence of the increasing evidence for the importance of competition among herbivorous insects is that its role in the evolution of the specialist lifestyle of herbivorous insects needs to be re-evaluated. It is likely that competition is more important than has been considered until recently.

11.7.3 Reduced mortality from natural enemies

Herbivorous insects have many enemies, such as arthropod predators and parasitoids or vertebrate predators. There are various examples of how plants enhance the effectiveness of the herbivore's enemies such as insect predators or parasitoids,[37] or even predatory birds.[88] However, herbivorous insects may exploit their host plant's defences to their own benefit, that is, in their defence against predators, pathogens, and parasitoids (see Chapter 10). For instance, plant secondary metabolites may be sequestered and exploited in the herbivore's defence against its enemies. To do so, they must have adapted to the plant's defence to avoid being negatively affected themselves. Specialist herbivores may be better able to escape from their natural enemies, such as generalist predators.[16] For instance, in a set of greenhouse experiments vespid predators selected more generalist caterpillars than specialized caterpillars.[12] Specialist herbivores also take less time to accept a host plant[15,42] and have adapted mouthparts that allow more efficient feeding, which could enhance the insect's chances of escaping from predators.[14] After all, during feeding from a host plant, the chances of falling victim to their enemies are much (up to 100 times) greater than when resting.[13] For instance, during extensive observations of *Uresiphita reversalis* caterpillars, the majority of the herbivores observed to be killed by anthocorid bugs were actually feeding, even when the proportion of time spent feeding was much less than the proportion spent resting.[13]

It has been argued that the reduced mortality from natural enemies in specialized insect herbivores may be the result rather than the cause of host-plant specialization.[75] However, this suggests that there is one single factor that causes herbivores to specialize; this is highly unlikely, given that herbivores are 'between the devil and the deep blue sea'.[83] Indeed, to date no single factor has been identified as the cause of host-plant specialization, whereas there is good evidence that several factors may play a role.[16,108,115] The relative contribution of the different factors is likely to depend on the system being considered.

11.7.4 Phylogenetic relationships

The evolution of host-plant choices can be investigated experimentally and has yielded interesting information on host-plant adaptation in herbivorous insects (see Section 11.5). However, it is more difficult to assess the evolution of host-plant choices over longer periods in evolutionary history as this cannot be based on fossils. A tool for obtaining such information is to make a cladogram or phylogenetic tree, which shows the sequence of divergence of extant species from a common ancestor. So far, few cladograms have been produced for herbivorous insects,[45,46] but the limited number that are available show that related insect species feed on related host-plant species[59] (Fig. 11.11): in the majority of insect groups investigated host-family shifts occurred in less than 17% of insect speciation events.[46]

Another way of looking at phylogenetic relationships is to assess the correspondence between insect and host-plant phylogenies. Four types of such relationship have been distinguished (Fig. 11.12).[74] In type A, closely related insect species live oligophagously or monophagously on distantly related plant species (incongruent phylogenies). For example, the European *Yponomeuta* species are narrowly specialized on host species of four plant families, which belong to three different plant orders.[95] Type B represents the situation in which closely related insect species live oligophagously (or partly monophagously) on closely related plant species (partly congruent cladograms). Typical examples are *Pieris brassicae, P. napi*, and *P. rapae*, which prefer roughly the same species of Brassicaceae.[24] In type C, closely related insect species live monophagously on closely related plant species and the cladograms are strongly congruent. An example of this is given for the chrysomelid genus *Phyllobrotica* and its host plants in Figure 11.13. In type D, a polyphagous species feeds on plant species that belong to different plant families. Types B and C suggest phylogenetic conservatism: that speciation in herbivorous insects is often accompanied by shifts between closely related plant taxa. Explicit investigations of the degree of matching in insect–plant phylogenies are scarce. An analysis of 14 assemblages for which at least partial phylogenies were available showed that in more than half of these assemblages the phylogeny correspondence was more than 0.5 on a scale of zero to one (Fig. 11.14).[46] However, the match was significant in only 25% of cases.[45] The phylogeny matchings of types B and C (see Fig. 11.11) can be considered to have phylogeny correspondence of far more than 0.5, whereas types A and D are expected to have phylogeny correspondence of less than 0.5 (Fig. 11.14). Recently, a new example of a congruent phylogeny has been presented for cerambycid beetles and *Asclepias*

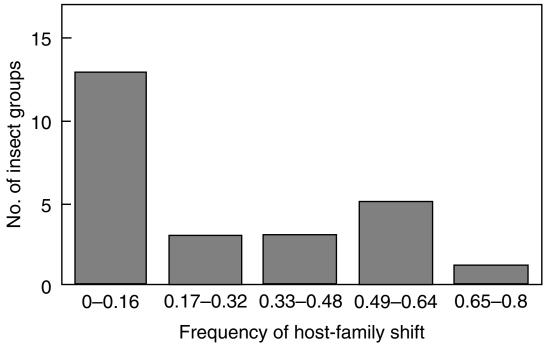

Figure 11.11 Frequency distribution of shifts in host family per speciation event in 25 herbivorous insect groups. (From Farrell *et al.*, 1992.[46] © American Institute of Biological Sciences.)

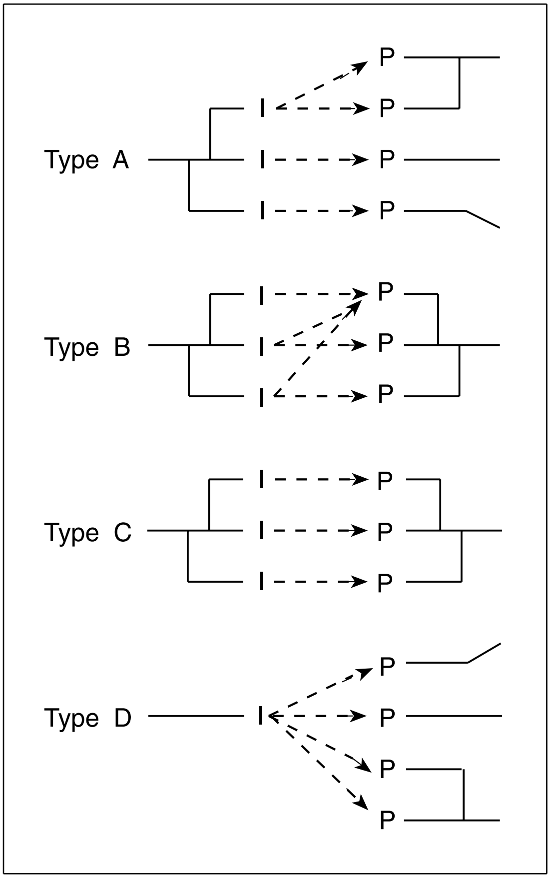

Figure 11.12 Types of cladogram between closely related insect species or a single insect species and their host plants. I, herbivorous insect species; P, host plant; broken lines with arrows indicate trophic relations. (Redrawn from Jermy, 1984.)[74]

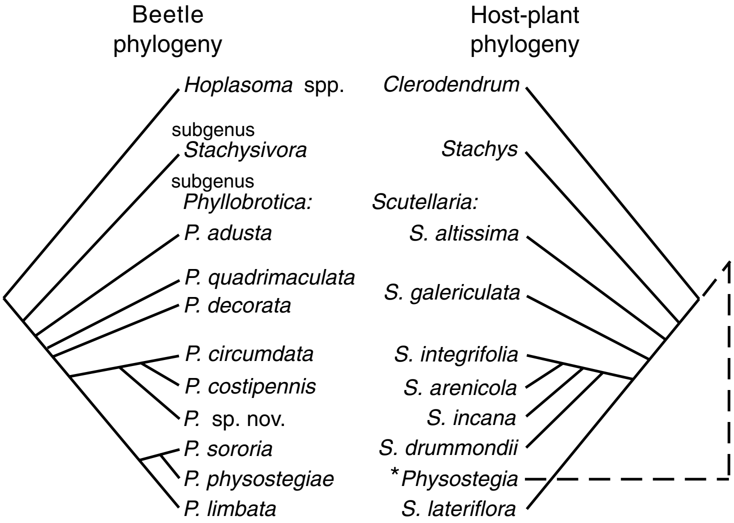

Figure 11.13 Cladograms of the chrysomelid genus *Phyllobrotica* (with the sister genus *Hoplasoma*) and of its host plants. Each insect taxon is placed opposite its host. Beetle species with unknown hosts and plant species that are not hosts to the *Phyllobrotica* lineage have been excluded. (From Farrell and Mitter, 1990.)[44]

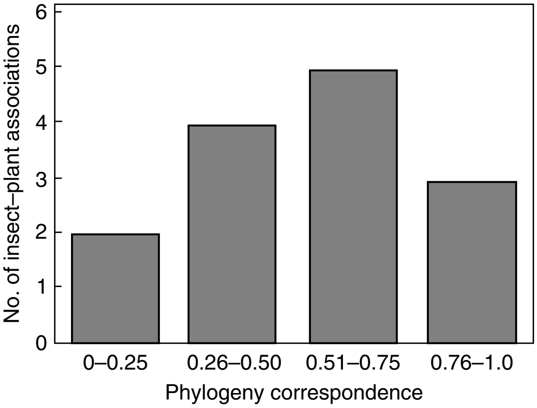

Figure 11.14 Frequency distribution of correspondence between the phylogenies of 14 independent insect groups and the phylogenies of their respective host-plant groups. The phylogeny correspondence is Colless' consensus index (see Ref. 44) and ranges from 0 (no correspondence) to 1 (complete correspondence). (Redrawn from Farrell *et al.*, 1992.[46] © American Institute of Biological Sciences.)

plants.[45] Thus, although the information is limited and close congruent phylogenesis is rare, there is a considerable proportion of partially congruent phylogenies. However, the number of studies on phylogeny matching is very small. Moreover, it is interesting to note that the best examples for congruent phylogenesis relate to beetles, where both adults and juveniles feed on plants and therefore have an intimate dependence on their hosts, in contrast to the situation in, for example, Lepidoptera. We should be careful, however, to conclude on the basis of congruent phylogenies alone that reciprocal evolution has occurred rather than, for instance, herbivores tracking some feature correlated with host phylogeny.

11.8 Reciprocal evolution of herbivorous insects and their host plants

In 1964 Ehrlich and Raven proposed that herbivorous insects and their host plants are involved in an arms race through reciprocal evolution, or co-evolution.[43] In the following decades, the concept of co-evolution has been subject of many discussions.[73,74,108,126] Co-evolution is defined as 'an evolutionary change in a trait of the individuals in one population in response to a trait of the individuals of a second population, followed by an evolutionary response by the second to the change in the first'.[73] The theory of co-evolution has stimulated many studies over recent decades. In a survey of members of the British Ecological Society in 1989, ecologists ranked co-evolution and animal–plant co-evolution among the 50 most important concepts in ecology.[125]

Different types of co-evolution have been recognized, such as (a) classical co-evolution in which reciprocal evolution between species pairs takes place,[43] (b) diffuse co-evolution in which co-evolution is considered in a community context rather than a two-species interaction,[50] and (c) the geographical mosaic theory of co-evolution that takes into account the spatial variation occurring within populations so that there is a continually shifting geographical pattern of co-evolution between two or more species.[125] According to the latter theory 'much of the dynamics of the coevolutionary process need not result eventually in an escalating series of adaptations and counter-adaptations that become fixed traits within species'.[125]

In the following paragraphs we first present criticism of the concept of co-evolution to explain the present insect–plant relationships, and subsequently provide data that support co-evolution.

11.8.1 Criticism of the theory of co-evolution

Several points of criticism have been raised to argue that co-evolution between insect herbivores and their host plants does not occur. The main arguments are that the partners (i.e. herbivorous insects and their host plants) are unequal partners that are involved in asymmetrical interactions in which the plants exert selection on the herbivores, but the herbivores do not exert (sufficient) selection on the plants for reciprocal selection to occur.[74,121] As a result, insects are considered not to influence the evolution of plants. For instance, the fact that insects are not abundantly present throughout the area where their host plant occurs is thought to imply that the selection pressure of the insect is not the same for all individuals of the species and would therefore be too weak to result in the development of resistance. The classical co-evolutionary theory of Ehrlich and Raven indeed describes co-evolution, especially at the species level.[43] However, evolutionary mechanisms do not operate at the species level but at the level of the individual. The critics of classical co-evolutionary theory are right to point out that the evidence in support of this theory is sparse. Although this may indicate that the theory is not generally applicable, it does not imply that co-evolution does not play a role at all in interactions between insect herbivores and their host plants (see Section 11.8.2).

Instead of co-evolution, the proponents of asymmetrical interactions between plants and insects propose that sequential evolution occurs, that is, the evolution of herbivorous insects follows the evolution of plants, but the reverse does not occur.[74] This theory has been put forward by Jermy,[74] and assumes that insects take advantage of the available niches that plants abundantly provide. Host-plant selection is assumed to be 'mainly a behavioral process which is governed primarily by the insect's chemosensory system. Therefore, the emergence of new insect–host plant relationships results most likely from evolutionary changes in the insects' chemosensory systems. Adaptation to the nutritional quality of the new host plant is a secondary process'.[74] This argument, however, does not incorporate the well known phenomenon that insect behaviour can be modified through associative learning (see Chapter 8). As a result, herbivores may learn to use certain cues to avoid a host plant that is toxic or otherwise nutritionally unsuitable. Thus, toxins and nutrients within the plant have a feedback mechanism that can influence host selection behaviour. The behaviour of an insect is a result of the interpretation of information coming from the chemoreceptors. Studies on associative learning show that an insect can respond to the same chemical information in opposite ways as a result of different previous experiences in the presence of this information. The same cue that results in attraction after having been presented in the presence of food may be avoided after having previously been associated with starvation.[41] Therefore, as long as cues from the plant can be perceived by the insect, the acceptance or rejection of it can be modified by other factors, such as toxicity or nutrient composition. Consequently, nutritional quality is a major component in the evolution of plant preference. An insect that happens to feed on a new plant without being intoxicated, and is able to recognize chemical cues from the plant, may use this information to find a food source that has proven to be suitable.

Admittedly, plants are exposed to natural selection by more than herbivorous insects alone. Microbial pathogens, mammalian herbivores, and abiotic stress are all expected to exert selection on plants. The theory of sequential selection has not indicated what is considered to be the main selection factor shaping plant evolution. A combination of factors is assumed to exert selection on plants.

The argument that plants influence the evolution of herbivorous insects rather than the reverse seems to conflict with palaeontological data that show that the familial diversification of angiosperm plants took place after the major familial diversification of insects.[81]

11.8.2 Support for the theory of co-evolution

Although the theory of co-evolution, as put forward by Ehrlich and Raven for the evolution of insect–plant relationships,[43] has met severe criticism, and support for the version as originally formulated may seem to be scant, it has motivated a wealth of studies into the selection pressures that shape insect–plant relationships. These studies have provided evidence in support of the theory at several levels of integration. Moreover, the theory of co-evolution itself has evolved over recent decades and has included the role of variation at the subspecies level. Reciprocal evolution can be found at several levels, even when many confounding factors may hinder the analysis. Below, we provide some of the supportive evidence as well as the problems connected with obtaining supportive evidence.

First, there is support at the species level. For instance, the cladograms of the chrysomelid genus *Phyllobrotica* and its host plants match: closely related insect species live monophagously on closely related plant species (see Fig. 11.13).[44] Only a few such cladograms have been reported.[44,45] However, it should be realized that the total number of studies on parallel cladograms is limited[46] and that cladogram matching is an extreme in which the traits have become fixed throughout the geographical ranges of the species.[125] Furthermore, a comparison of independent molecular clocks for a lineage of chrysomelid beetles in the genus *Blepharida* and their burseraceous host plants showed that the plants' defences and the insects' counter-defensive feeding traits evolved roughly in synchrony over a period of more than 100 million years.[7]

Second, support can be found at the level of populations. An analysis of different populations of wild parsnip (*Pastinaca sativa*) and its specialist herbivorous insect the parsnip webworm (*Depressaria pastinacella*) showed that the plant populations were polymorphic with four major phenotypes related to furanocoumarin composition occurring. The herbivore populations also consisted of different phenotypes with respect to their ability to metabolize the different types of furanocoumarin. Thus, there is spatial variation in both plant and herbivore. Moreover, there was a remarkable degree of frequency matching in three out of four of the populations when considering plant and herbivore phenotype clusters (Fig. 11.15).[8]

A meta-analysis of the floristic distribution and toxicity of phytochemicals to herbivorous insects on the one hand and of herbivore specialization on the other, provides support for the 'escape and radiation' prediction of Ehrlich and Raven's theory:[43] secondary metabolites with a narrow distribution, representing newly evolved metabolites, are more toxic than metabolites with a wider distribution, representing metabolites that evolved longer ago.[27] This extensive analysis of the phytochemical literature provides important support for the co-evolutionary theory.

Processes at the individual and population level should be the primary focus when investigating evolutionary dynamics, as selection acts on individuals rather than species. This is exactly what Thompson did when putting forward the geographical mosaic of co-evolution.[125] This theory states that the co-evolutionary process is more dynamic than is apparent from the study of individual populations or the distribution of characters found in phylogenetic trees. After all, the distributions of a herbivore species and its host plant do not by definition overlap and a host plant can use different ways to escape from its herbivore, including the colonization of (micro)habitats that are not (yet) suitable for the herbivore. It is well established that plant and insect populations can form meta-populations (i.e. assemblages of local subpopulations with local adaptations).[31,62] This

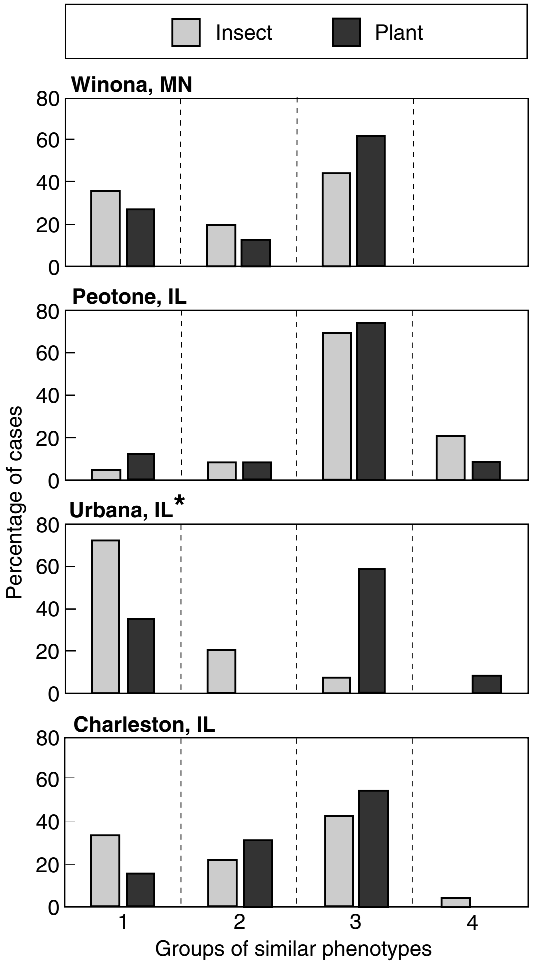

Figure 11.15 Phenotype frequency distributions of insects (parsnip webworm, *Depressaria pastinacella*) and plants (wild parsnip, *Pastinaca sativa*) for each of the four populations. Each of the four groups of phenotype represents phenotypes that are similar with respect to production of furanocoumarins (wild parsnip plants) or detoxification of parsnip furanocoumarins (parsnip webworm herbivores). The plant samples collected in Winona, Peotone, and Charleston were taken in close proximity to the collected herbivore pupae. In contrast, the insect samples collected in Urbana were from a 12-hectare area and the plants originated from a much smaller area (0.3 ha) not directly linked to the insect pupae. *Denotes a significant mismatch between plant and insect patterns. (Redrawn from Berenbaum and Zangerl, 1998.[8] © 1998 National Academy of Sciences, USA.)

can affect migration, selection, and local adaptation so that an analysis of reciprocal adaptation carried out at the species level may not be representative of the level at which the adaptation actually occurs. In addition to addressing the individual rather than the population or the species, the individual's phenotype is inherently plastic. Individuals that interact may adjust their phenotype in response to their respective partner. This can reflect an evolutionary response to variation as encountered by individuals.[1] Although phenotypic plasticity such as induced plant defence has been considered to 'simply reflect alterations in plant metabolism under stress conditions resulting in changes in plant chemistry which the insect cannot tolerate',[74] it has recently been hypothesized as potentially leading to 'reciprocal change in ecological time, altered community patterns, and expanded evolutionary potential of species'.[1]

Third, mathematical modelling has shown that arms races through co-evolutionary cycles are possible. The models that have been constructed are based on key assumptions such as costs and benefits of resistance in the plant, and how costs of virulence or detoxification ability in the herbivore change with levels of these traits.[10] However, the modelling and experimental studies have not been coordinated and this may be one of the reasons why there is a poor support of the models by experimental data.

In conclusion, the data in support of classical co-evolution may seem scant. However, this may in part be explained by the fact that co-evolution has often been investigated at levels of integration too far above the level of the individual, which is the basic element under natural selection.

Traditionally, co-evolution has been considered in a bitrophic context, related to direct plant defence. The second major form of plant defence, indirect defence, is a mutualism between the plant and the natural enemies of the herbivore,[35,37,110] and mutualism has been considered more likely to evolve through co-evolution than antagonistic interactions.[125] However, even though both the plant and the enemy of the herbivore benefit from indirect defence such as herbivore-induced plant volatiles, it is unlikely that such volatiles developed from the very beginning as an indirect defence.[35,110] Most likely their original function was a different one, and subsequent selection from carnivorous arthropods has probably moulded the characteristic of induced plant volatiles. The induced blend can be specific for the herbivore that infests the plant, and carnivorous insects can use the blends to discriminate between herbivore species that induce them.[38] This specific response of plants supports the hypothesis that plant defence is under the selection of herbivorous insects. The responses by carnivorous arthropods provide selective benefits in terms of reproductive success.[56,132] However, in a game-theoretical framework it has been hypothesized that defensive characteristics may boomerang as a result of changing responses in neighbouring competitors.[110] After all, defences not only protect the organism that exhibits the defence characteristic but may also contribute to the protection of their neighbours.[36,110] This may contribute to variation in defence expression and may explain why some plants invest relatively little in direct as well as indirect defences.[110]

In conclusion, the theory of co-evolution has yielded an intense debate on the basic evolutionary process that influences insect–plant interactions. This debate continues critically to analyse new evidence brought into the scientific arena and is likely to stimulate further research into the evolution of the contest between plants and herbivorous insects and the role of chemical weapons, allies, and refuges.

11.9 Conclusions

As sessile organisms, plants have to deal with a wide range of abiotic and biotic stresses. Their omnipresence on our planet shows that they are well able to deal with these diverse stresses. The search for the selective force that is most important in plant evolution has yielded a lively discussion. Although there is ample evidence that herbivorous insects adapt to plants, the evidence that plants adapt to insect herbivores is scant. It has often been considered that insect–plant interactions are characterized by asymmetrical selection pressures. However, there are ample indications that the selective pressure of insects on their host plants has been underestimated. Especially at the individual level and in the micro-evolutionary timescale, reciprocal selective forces have been reported and hypotheses on these are important in guiding experimental research. It is expected that the debate on co-evolution, and consequently the experimental approach addressing reciprocal selection, will continue in the decades to come. The geographical mosaic theory of co-evolution that stresses spatial variation in evolutionary processes, is likely to play an important role. For the identification of spatially variable processes, molecular techniques will prove to be highly valuable. The mechanisms underlying the synchronous evolution of insects and plants, which together comprise some of the richest assemblages in terrestrial ecosystems, will remain an important theme of study. An understanding of the evolution of insect–plant interactions will contribute to our understanding of the origins of biodiversity and to the whole of biology.

11.10 References

1. Agrawal, A.A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science*, **294**, 321–6.
2. Agrawal, A.A. and Karban, R. (1999). Why induced defenses may be favored over constitutive strategies in plants. In *The ecology and evolution of inducible defenses* (ed. R. Tollrian and C.D. Harvell), pp. 45–61. Princeton University Press, Princeton.
3. Agrawal, A.A. and Kurashige, N.S. (2003). A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae. Journal of Chemical Ecology*, **29**, 1403–15.
4. Asaoka, K. (2000). Deficiency of gustatory sensitivity to some deterrent compounds in 'polyphagous' mutant strains of the silkworm, *Bombyx mori. Journal of Comparative Physiology A*, **186**, 1011–18.
5. Avison, T.I. (1988). Cited in Ref. 117.
6. Baldwin, I.T. (1998). Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proceedings of the National Academy of Sciences of the USA*, **95**, 8113–18.
7. Becerra, J.X. (2003). Synchronous coadaptation in an ancient case of herbivory. *Proceedings of the National Academy of Sciences of the USA*, **100**, 12804–7.
8. Berenbaum, M.R. and Zangerl, A.R. (1998). Chemical phenotype matching between a plant and its insect herbivore. *Proceedings of the National Academy of Sciences of the USA*, **95**, 13743–8.
9. Berenbaum, M.R., Zangerl, A.R., and Nitao, J.K. (1986). Constraints on chemical coevolution: wild parsnips and the parsnib webworm. *Evolution*, **40**, 1215–28.

10. Bergelson, J., Dwyer, G., and Emerson, J.J. (2001). Models and data on plant–enemy coevolution. *Annual Review of Genetics*, **35**, 469–99.
11. Berlocher, S.H. and Feder, J.L. (2002). Sympatric speciation in phytophagous insects: moving beyond controversy? *Annual Review of Entomology*, **47**, 773–815.
12. Bernays, E.A. (1989). Host range in phytophagous insects: the potential role of generalist predators. *Evolutionary Ecology*, **3**, 299–311.
13. Bernays, E.A. (1997). Feeding by lepidopteran larvae is dangerous. *Ecological Entomology*, **22**, 121–3.
14. Bernays, E.A. (1998). Evolution of feeding behavior in insect herbivores—success seen as different ways to eat without being eaten. *BioScience*, **48**, 35–44.
15. Bernays, E.A. (2001). Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annual Review of Entomology*, **46**, 703–27.
16. Bernays, E. and Graham, M. (1988). On the evolution of host specificity in phytophagous arthropods. *Ecology*, **69**, 886–92.
17. Boller, E.F., Russ, K., Vallo, V., and Bush, G.L. (1976). Incompatible races of european cherry fruit fly, *Rhagoletis cerasi* (Diptera-Tephritidae), their origin and potential use in biological control. *Entomologia Experimentalis et Applicata*, **20**, 237–47.
18. Bush, G.L. (1969). Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). *Evolution*, **23**, 237–51.
19. Bush, G.L. (1975). Sympatric speciation in phytophagous parasitic insects. In *Evolutionary strategies of parasitic insects and mites* (ed. P.W. Price), pp. 187–206. Plenum, New York.
20. Bush, G.L. (1987). Evolutionary behaviour genetics. In *Evolutionary genetics of invertebrate behavior* (ed. M.D. Huettel), pp. 1–5. Plenum, New York.
21. Caillaud, M.C. and Via, S. (2000). Specialized feeding behavior influences both ecological specialization and assortative mating in sympatric host races of pea aphids. *American Naturalist*, **156**, 606–21.
22. Carson, H.L. (1987). Colonization and speciation. In *Colonization, succession and stability* (ed. A.J. Gray, M.J. Crawley, and P.J. Edwards), pp. 187–206. Blackwell Scientific, Oxford.
23. Chew, F.S. (1977). Coevolution of pierid butterflies and their cruciferous foodplants. 2. Distribution of eggs on potential foodplants. *Evolution*, **31**, 568–79.
24. Chew, F.S. and Renwick, J.A.A. (1995). Host plant choice in *Pieris* butterflies. In *Chemical ecology of insects* (ed. R.T. Cardé and W.J. Bell), pp. 214–38. Chapman & Hall, New York.
25. Cipollini, D. (2002). Variation in the expression of chemical defenses in *Alliaria petiolata* (Brassicaceae) in the field and common garden. *American Journal of Botany*, **89**, 1422–30.
26. Claridge, M.F. (1995). Species concepts and speciation in insect herbivores. Planthopper case-studies. *Bollettino di Zoologia*, **62**, 53–8.
27. Cornell, H.V. and Hawkins, B.A. (2003). Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. *American Naturalist*, **161**, 507–22.
28. Crawley, M.J. (1985). Reduction of oak fecundity by low-density herbivore populations. *Nature*, **314**, 163–4.
29. Damman, H. (1993). Patterns of interaction among herbivore species. In *Caterpillars. Ecological and evolutionary constraints on foraging* (ed. N.E. Stamp and T.M. Casey), pp. 132–69. Chapman & Hall, New York.
30. Darwin, C., (1859). *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. Murray, London.
31. De Jong, P.W. and Nielsen, J.K. (2002). Host plant use of *Phyllotreta nemorum*: do co-adapted gene complexes play a role? *Entomologia Experimentalis et Applicata*, **104**, 207–15.
32. Denholm, I., Devine, G.J., and Williamson, M.S. (2002). Evolutionary genetics. Insecticide resistance on the move. *Science*, **297**, 2222–3.
33. Denno, R.F., McClure, M.S., and Ott, J.R. (1995). Interspecific interaction in phytophagous insects: competition reexamined and resurrected. *Annual Review of Entomology*, **40**, 297–331.
34. Denno, R.F., Peterson, M.A., Gratton, C., Cheng, J.A., Langellotto, G.A., Huberty, A.F., *et al*. (2000). Feeding-induced changes in plant quality mediate interspecific competition between sap-feeding herbivores. *Ecology*, **81**, 1814–27.
35. Dicke, M. and Sabelis, M.W. (1989). Does it pay plants to advertize for bodyguards? Towards a cost–benefit analysis of induced synomone production. In *Causes and consequences of variation in growth rate and productivity of higher plants* (ed. H. Lambers, *et al*.), pp. 341–358. SPB Publishing, The Hague.
36. Dicke, M. and Vet, L.E.M. (1999). Plant–carnivore interactions: evolutionary and ecological consequences for plant, herbivore and carnivore. In *Herbivores: between plants and predators* (ed. H. Olff, V.K. Brown, and R.H. Drent), pp. 483–520. Blackwell Science, Oxford.
37. Dicke, M. and Van Loon, J.J.A. (2000). Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata*, **97**, 237–49.

38. Dicke, M., Van Poecke, R.M.P., and De Boer, J.G. (2003). Inducible indirect defence of plants: from mechanisms to ecological functions. *Basic and Applied Ecology*, **4**, 27–42.
39. Dicke, M., Van Loon, J.J.A., and De Jong, P.W. (2004). Ecogenomics benefits community ecology. *Science*, **305**, 618–19.
40. Dobler, S., Mardulyn, P., Pasteels, J.M., and Rowell-Rahier, M. (1996). Host-plant switches and the evolution of chemical defense and life history in the leaf beetle genus *Oreina*. *Evolution*, **50**, 2373–86.
41. Drukker, B., Bruin, J., and Sabelis, M.W. (2000). Anthocorid predators learn to associate herbivore-induced plant volatiles with presence or absence of prey. *Physiological Entomology*, **25**, 260–5.
42. Dukas, R. (2004). Causes and consequences of limited attention. *Brain Behavior and Evolution*, **63**, 197–210.
43. Ehrlich, P.R. and Raven, P.H. (1964). Butterflies and plants: a study in coevolution. *Evolution*, **18**, 586–608.
44. Farrell, B. and Mitter, C. (1990). Phylogenesis of insect/plant interactions: have *Phyllobrotica* leaf beetles (Chrysomelidae) and the lamiales diversified in parallel? *Evolution*, **44**, 1389–403.
45. Farrell, B.D. and Mitter, C. (1998). The timing of insect/plant diversification: might *Tetraopes* (Coleoptera: Cerambycidae) and *Asclepias* (Asclepiadaceae) have co-evolved? *Biological Journal of the Linnean Society*, **63**, 553–77.
46. Farrell, B.D., Mitter, C., and Futuyma , D.J. (1992). Diversification at the insect–plant interface. *BioScience*, **42**, 34–42.
47. Feder, J.L., Chilcote, C.A., and Bush, G.L. (1990a). Regional, local and microgeographic allele frequency variation between apple and hawthorn populations of *Rhagoletis pomonella* in western Michigan. *Evolution*, **44**, 595–608.
48. Feder, J.L., Chilcote, C.A., and Bush, G.L. (1990b). The geographic pattern of genetic differentation between host associated populations of *Rhagoletis pomonella* (Diptera: Tephritidae) in the eastern United States and Canada. *Evolution*, **44**, 570–94.
49. Fine, P.V.A., Mesones, I., and Coley, P.D. (2004). Herbivores promote habitat specialization by trees in Amazonian forests. *Science*, **305**, 663–5.
50. Fox, L.R. (1988). Diffuse coevolution within complex communities. *Ecology*, **69**, 906–7.
51. Fox, L.R. and Morrow, P.A. (1981). Specialization: species property or local phenomenon? *Science*, **211**, 887–93.
52. Fraenkel, G.S. (1959). The raison d'être of secondary plant substances. *Science*, **129**, 1466–70.
53. Frey, J.E. and Bush, G.L. (1990). *Rhagoletis* sibling species and host races differ in host odor recognition. *Entomologia Experimentalis et Applicata*, **57**, 123–31.
54. Frey, J.E. and Bush, G.L. (1996). Impaired host odor perception in hybrids between the sibling species *Rhagoletis pomonella* and *R. mendax*. *Entomologia Experimentalis et Applicata*, **80**, 163–5.
55. Frey, J.E., Bierbaum, T.J., and Bush, G.L. (1992). Differences among sibling species *Rhagoletis mendax* and *R. pomonella* (Diptera, Tephritidae) in their antennal sensitivity to host fruit compounds. *Journal of Chemical Ecology*, **18**, 2011–24.
56. Fritzsche-Hoballah, M.E. and Turlings, T.C.J. (2001). Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. *Evolutionary Ecology Research*, **3**, 553–65.
57. Fry, J.D. (1989). Evolutionary adaption to host plants in a laboratory population of the phytophagous mite *Tetranychus urticae* Koch. *Oecologia*, **81**, 559–65.
58. Funk, D.J. and Bernays, E.A. (2001). Geographic variation in host specificity reveals host range evolution in *Uroleucon ambrosiae* aphids. *Ecology*, **82**, 726–39.
59. Futuyma, D.J. and Mitter, C. (1996). Insect–plant interactions: the evolution of component communities. *Philosophical Transactions of the Royal Society of London, Series B*, **351**, 1361–6.
60. Gardner, S.N. and Agrawal, A.A. (2002). Induced plant defence and the evolution of counter-defences in herbivores. *Evolutionary Ecology Research*, **4**, 1131–51.
61. Glawe, G.A., Zavala, J.A., Kessler, A., Van Dam, N.M., and Baldwin, I.T. (2003). Ecological costs and benefits correlated with trypsin protease inhibitor production in *Nicotiana attenuata*. *Ecology*, **84**, 79–90.
62. Hanski, I. (1999). *Metapopulation ecology*. Oxford University Press, Oxford.
63. Hanson, F.E. (1976). Comparative studies on induction of food preferences in lepidopterous larvae. *Symposia Biologica Hungarica*, **16**, 71–7.
64. Ho, T.H. (1965). The life history and control of the diamondback moth in Malaya. *Ministry of Agriculture Co-operation Bulletin*, **118**, 1–26.
65. Hoballah, M.E., Kollner, T.G., Degenhardt, J., and Turlings, T.C.J. (2004). Costs of induced volatile production in maize. *Oikos*, **105**, 168–80.
66. Hsiao, T.H. and Hsiao, C. (1985). Hybridization and cytoplasmic incompatibility among alfalfa weevil strains. *Entomologia Experimentalis et Applicata*, **37**, 155–9.
67. Huettel, M.D. and Bush, G.L. (1972). The genetics of host selection and its bearing on sympatric speciation in *Procecidochares* (Diptera: Tephritidae). *Entomologia Experimentalis et Applicata*, **15**, 465–80.

68. Jaenike, J. (1985). Genetic and environmental determinants of food preference in *Drosophila tripunctata*. *Evolution*, **39**, 362–9.
69. Jaenike, J. (1987). Genetics of oviposition preference in *Drosophila tripunctata*. *Heredity*, **59**, 363–9.
70. Janz, N. (1998). Sex-linked inheritance of host-plant specialization in a polyphagous butterfly. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **265**, 1675–8.
71. Janz, N., Nyblom, K., and Nylin, S. (2001). Evolutionary dynamics of host-plant specialization: a case study of the tribe Nymphalini. *Evolution*, **55**, 783–96.
72. Janzen, D.H. (1974). Tropical blackwater rivers, animals, and mast fruiting by the Dipterocarpaceae. *Biotropica*, **6**, 69–103.
73. Janzen, D.H. (1980). When is it coevolution? *Evolution*, **34**, 611–12.
74. Jermy, T. (1984). Evolution of insect host plant relationships. *American Naturalist*, **124**, 609–30.
75. Jermy, T. (1988). Can predation lead to narrow food specialization in phytophagous insects? *Ecology*, **69**, 902–4.
76. Karban, R. and Agrawal, A.A. (2002). Herbivore offense. *Annual Review of Ecology and Systematics*, **33**, 641–64.
77. Kennedy, G.G. and Barbour, J.D. (1992). Resistance variation in natural and managed systems. In *Plant resistance to herbivores and pathogens. Ecology, evolution and genetics* (ed. R.S. Fritz and E.L. Simms), pp. 13–41. University of Chicago Press, Chicago.
78. Kessler, A., Halitschke, R., and Baldwin, I.T. (2004). Silencing the jasmonate cascade: induced plant defenses and insect populations. *Science*, **305**, 665–8.
79. Klun, J.A. and Maini, S. (1979). Genetic basis of an insect chemical communication system: the European corn borer (Lepidoptera, Pyralidae). *Environmental Entomology*, **8**, 423–6.
80. Labandeira, C.C. (1998). Early history of arthropod and vascular plant associations. *Annual Review of Earth and Planetary Sciences*, **26**, 329–77.
81. Labandeira, C.C. and Sepkoski, J.J. (1993). Insect diversity in the fossil record. *Science*, **261**, 310–15.
82. Labandeira, C.C., Johnson, K.R., and Wilf, P. (2002). Impact of the terminal Cretaceous event on plant-insect associations. *Proceedings of the National Academy of Sciences of the USA*, **99**, 2061–6.
83. Lawton, J.H. and McNeill, S. (1979) Between the devil and the deep blue sea: on the problem of being a herbivore. In *Population dynamics* (ed. R.M. Anderson, B.D. Turner, and L.R. Taylor), pp. 223–44. Blackwell, Oxford.
84. Leslie, J.F. and Dingle, H. (1983). A genetic basis of oviposition preference in the large milkweed bug, *Oncopeltus fasciatus*. *Entomologia Experimentalis et Applicata*, **34**, 215–20.
85. Louda, S.M. and Potvin, M.A. (1995). Effect of inflorescence feeding insects on the demography and lifetime fitness of a native plant. *Ecology*, **76**, 229–45.
86. Louda, S.M. and Rodman, J.E. (1996). Insect herbivory as a major factor in the shade distribution of a native crucifer (*Cardamine cordifolia* A. Gray, bittercress). *Journal of Ecology*, **84**, 229–37.
87. Mägdefrau, K. (1959). *Vegetationsbilder der Vorzeit* (3rd edn). G. Fischer, Jena.
88. Mantyla, E., Klemola, T., and Haukioja, E. (2004). Attraction of willow warblers to sawfly-damaged mountain birches: novel function of inducible plant defences? *Ecology Letters*, **7**, 915–18.
89. Marquis, R.J. (2004). Herbivores rule. *Science*, **305**, 619–21.
90. Marshall, J.L. (2004). The *Allonemobius–Wolbachia* host–endosymbiont system: evidence for rapid speciation and against reproductive isolation driven by cytoplasmic incompatibility. *Evolution*, **58**, 2409–25.
91. Mauricio, R. and Rausher, M.D. (1997). Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution*, **5**, 1435–44.
92. May, R.M. (1981). *Theoretical ecology: principles and applications*. Blackwell, Oxford.
93. Mayr, E. (1954). Change of genetic environment and evolution. In *Evolution as a process* (ed. J. Huxley, A.C. Hardy, and E.B. Ford), pp. 157–80. Allen & Unwin, London.
94. Mayr, E. (2004). 80 years of watching the evolutionary scenery. *Science*, **305**, 46–7.
95. Menken, S.B.J., Herrebout, W.M., and Wiebes, J.T. (1992). Small ermine moths (*Yponomeuta*): their host relations and evolution. *Annual Review of Entomology*, **37**, 41–88.
96. Michel, A., Arias, R.S., Scheffler, B.E., Duke, S.O., Netherland, M., and Dayan, F.E. (2004). Somatic mutation-mediated evolution of herbicide resistance in the nonindigenous invasive plant hydrilla (*Hydrilla verticillata*). *Molecular Ecology*, **13,** 3229–37.
97. Mitter, C., Farrell, B., and Wiegmann, B. (1988). The phylogenetic study of adaptive zones—has phytophagy promoted insect diversification? *American Naturalist*, **132**, 107–28.
98. Mopper, S. (1996). Adaptive genetic structure in phytophagous insect populations. *Trends in Ecology and Evolution*, **11**, 235–8.

99. Morin, P.A., Luikart, G., and Wayne, R.K. (2004). SNPs in ecology, evolution and conservation. *Trends in Ecology and Evolution*, **19**, 208–16.
100. Müller, F.P. (1985). Genetic and evolutionary aspects of host choice in phytophagous insects, especially aphids. *Biologisches Zentralblatt*, **104**, 225–37.
101. Ng, D. (1988). A novel level of interactions in plant–insect systems. *Nature*, **334**, 611–13.
102. Nielsen, J.K. and De Jong, P.W. (2005). Temporal and host-related variation in frequencies of genes that enable *Phyllotreta nemorum* to utilize a novel host plant, *Barbarea vulgaris*. *Entomologia Experimentalis et Applicata*, **115**, 265–70.
103. Panda, N. and Khush, G.S. (1995). *Host plant resistance to insects*. CABI, Wallingford.
104. Pappers, S.M., Van der Velde, G., Ouborg, N.J., and Van Groenendael, J.M. (2002). Genetically based polymorphisms in morphology and life history associated with putative host races of the water lily leaf beetle, *Galerucella nymphaeae*. *Evolution*, **56**, 1610–21.
105. Pelozuelo, L., Malosse, C., Genestier, G., Guenego, H., and Frerot, B. (2004). Host-plant specialization in pheromone strains of the European corn borer *Ostrinia nubilalis* in France. *Journal of Chemical Ecology*, **30**, 335–52.
106. Preston, C.A., Laue, G., and Baldwin, I.T. (2001). Methyl jasmonate is blowing in the wind, but can it act as a plant–plant airborne signal? *Biochemical Systematics and Ecology*, **29**, 1007–23.
107. Ratzka, A., Vogel, H., Kliebenstein, D.J., Mitchell-Olds,T., and Kroymann, J. (2002). Disarming the mustard oil bomb. *Proceedings of the National Academy of Sciences of the USA*, **99**, 11223–8.
108. Rausher, M.D. (1988). Is coevolution dead? *Ecology*, **69**, 898–901.
109. Rodriguez, R.L., Sullivan, L.E., and Cocroft, R.B. (2004). Vibrational communication and reproductive isolation in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Evolution*, **58**, 571–8.
110. Sabelis, M.W., Van Baalen, M., Bakker, F.M., Bruin, J., Drukker, B., Egas, M., *et al*. (1999). The evolution of direct and indirect plant defence against herbivorous arthropods. In *Herbivores: between plants and predators* (ed. H. Olff, V.K. Brown, and R.H. Drent), pp. 109–66. Blackwell, Oxford.
111. Scheirs, J. and De Bruyn, L. (2002). Integrating optimal foraging and optimal oviposition theory in plant–insect research. *Oikos*, **96**, 187–91.
112. Scheirs, J., De Bruyn, L., and Verhagen, R. (2000). Optimization of adult performance determines host choice in a grass miner. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **267**, 2065–9.
113. Schilthuizen, M. (2001). *Frogs, flies and dandelions: the making of species*. Oxford University Press. Oxford.
114. Schneider, J.C. and Roush, R.T. (1986). Genetic differences in oviposition preference between two populations of *Heliothis virescens*. In *Evolutionary genetics of invertebrate behavior* (ed. M.D. Huettel), pp. 163–71. Plenum, New York.
115. Schultz, J.C. (1988). Many factors influence the evolution of herbivore diets, but plant chemistry is central. *Ecology*, **69**, 896–7.
116. Scriber, J.M., Giebink, B.L., and Snider, D. (1991). Reciprocal latitudinal clines in oviposition behavior of *Papilio glaucus* and *P. canadensis* across the Great Lakes hybrid zone: possible sex-linkage of oviposition preferences. *Oecologia*, **87**, 360–8.
117. Sheck, A.L. and Gould, F. (1993). The genetic basis of host range in *Heliothis virescens*: larval survival and growth. *Entomologia Experimentalis et Applicata*, **69**, 157–72.
118. Simms, E.L. and Rausher, M.D. (1989). The evolution of resistance to herbivory in *Ipomoea purpurea*. II. Natural selection by insects and costs of resistance. *Evolution*, **43**, 573–85.
119. Steppuhn, A., Gase, K., Krock, B., Halitschke, R., and Baldwin, I.T. (2004). Nicotine's defensive function in nature. *PLoS Biology*, **2**, e217.
120. Stride, G.O. and Straatman, R. (1962). The host plant relationship of an Australian swallowtail *Papilio augeus*, and its significance in the evolution of host plant selection. *Proceedings of the Linnean Society of New South Wales*, **87**, 69–78.
121. Strong, D.R., Lawton, J.H., and Southwood, T.R.E. (1984). *Insects on plants: community patterns and mechanisms*. Harvard University Press, Cambridge, MA.
122. Tabashnik, B.E. (1986). Evolution of host utilization in *Colias* butterflies. In *Evolutionary genetics of invertebrate behavior* (ed. M.D. Huettel), pp. 173–84. Plenum Press, New York.
123. Thompson, J.N. (1987). Symbiont-induced speciation. *Biological Journal of the Linnean Society*, **32**, 385–93.
124. Thompson, J.N. (1988). Evolutionary genetics of oviposition preference in swallowtail butterflies. *Evolution*, **42**, 1223–34.
125. Thompson, J.N. (1994). *The coevolutionary process*. University of Chicago Press, Chicago.
126. Thompson, J.N. (1999). Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist*, **153**, S1–S14.

127. Thompson, J.N. and Pellmyr, O. (1991). Evolution of oviposition behavior and host preference in Lepidoptera. *Annual Review of Entomology*, **36**, 65–89.
128. Thompson, J.N., Wehling, W., and Podolsky, R. (1990). Evolutionary genetics of host use in swallowtail butterflies. *Nature*, **344**, 148–50.
129. Tooker, J.F., Koenig, W.A., and Hanks, L.M. (2002). Altered host plant volatiles are proxies for sex pheromones in the gall wasp *Antistrophus rufus*. *Proceedings of the National Academy of Sciences of the USA*, **99**, 15486–91.
130. Van der Putten, W.H., Vet, L.E.M., Harvey, J.A., and Wäckers, F.L. (2001). Linking above- and below-ground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology and Evolution*, **16**, 547–54.
131. Van Drongelen, W. and Van Loon, J.J.A. (1980). Inheritance of gustatory sensitivity in F1 progeny of crosses between *Yponomeuta cagnagellus* and *Yponomeuta malinellus* (Lepidoptera). *Entomologia Experimentalis et Applicata*, **28**, 199–203.
132. Van Loon, J.J.A., De Boer, J.G., and Dicke, M. (2000). Parasitoid–plant mutualism: parasitoid attack of herbivore increases plant reproduction. *Entomologia Experimentalis et Applicata*, **97**, 219–27.
133. Via, S. (1990). Ecological genetics and host adaptation in herbivorous insects: the experimental study of evolution in natural and agricultural systems. *Annual Review of Entomology*, **35**, 421–46.
134. Vrieling, K., Smit, W., and Van der Meijden, E. (1991). Tritrophic interactions between aphids (*Aphis jacobaeae* Schrank), ant species, *Tyria jacobaeae* L. and *Senecio jacobaea* L. lead to maintenance of genetic variation in pyrrolizidine alkaloid concentration. *Oecologia*, **86**, 177–82.
135. Waldvogel, M. and Gould, F. (1990). Variation in oviposition preference of *Heliothis virescens* in relation to macroevolutionary patterns of heliothine host range. *Evolution*, **44**, 1326–37.
136. Wasserman, S.S. and Futuyma, D.J. (1981). Evolution of host plant utilization in laboratory populations of the southern cowpea weevil *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). *Evolution*, **35**, 605–17.
137. Weeks, A.R., Reynolds, K.T., Hoffmann, A.A., and Mann, H. (2002). *Wolbachia* dynamics and host effects: what has (and has not) been demonstrated? *Trends in Ecology and Evolution*, **17**, 257–62.
138. Whittaker, R.H. (1977). Evolution of species diversity in land communities. *Evolutionary Biology*, **10**, 1–67.
139. Williams, K.S. and Simon, C. (1995). The ecology, behavior, and evolution of periodical cicadas. *Annual Review of Entomology*, **40**, 269–95.
140. Wittstock, U., Agerbirk, N., Stauber, E.J., Olsen, C.E., Hippler, M., Mitchell-Olds, T., *et al*. (2004). Successful herbivore attack due to metabolic diversion of a plant chemical defense. *Proceedings of the National Academy of Sciences of the USA*, **101**, 4859–64.
141. Wood, T.K. (1993). Diversity in the New World Membracidae. *Annual Review of Entomology*, **38**, 409–35.

CHAPTER 12

Insects and flowers: mutualism par excellence

When C. K. Sprengel, rector of a Lutheran school in Germany, became depressed as a result of his duties, his doctor advised him to study Nature and, to facilitate his recovery, taught his patient some elementary botany. Sprengel then gained an in-depth knowledge of flower morphology, nectar secretion and its function, and published in 1793 a treatise under the imaginative title *Das entdeckte Geheimniss der Natur im Bau und in der Befruchtung der Blumen* (*The Secret of Nature revealed in the Structure and Fertilization of Flowers*; Fig. 12.1).[116]

In this landmark book he demonstrated on the basis of observations on more than 500 plant species that, although most angiosperm flowers are hermaphroditic, they usually require pollinating insects in order to set seed, and concluded (p. 43) that 'so scheint die Natur es nicht haben zu wollen, dasz irgend eine Blume durch ihren eigenen Staub befruchtet werden solle.'* This view conflicted with the general belief at that time that seed-setting results from self-fertilization. Because contemporary botanists considered Sprengel as a non-professional, the work was ignored and remained in oblivion until, after a dormancy of more than 60 years, it came to the attention of Charles Darwin.[128] Whereas Sprengel did not ask why nature would not allow self-fertilization, Darwin did pose the question and pondered deeply about the biological meaning of cross-fertilization. Stimulated by Sprengel's observations he realized that cross-fertilization increases variation, thereby

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* 'nature does not seem to allow any flower to be fertilized by its own pollen'. Darwin expressed this notion in even more penetrating words when writing: 'Nature thus tells us, in the most emphatic manner, that she abhors perpetual self-fertilisation.' (*On the Various Contrivances by which British and Foreign Orchids are Fertilised by Insects, and on the Good Effects of Intercrossing*. John Murray, London, 1862).

Das

entdeckte Geheimniſs

der

NATUR

im Bau und in der Befruchtung

der

Blumen

von

CHRISTIAN KONRAD SPRENGEL,

Mit 25 Kupfertafeln.

Berlin, 1793.

bei Friedrich Vieweg dem ælteren.

C. Jäck Scripsit et Sculpsit

Gezeichnet v. C.K. Sprengel

W. Arndt Sculp.

Figure 12.1 Title page of C.K. Sprengel's classic book, which describes the role of insects in pollination.

forming the substrate for natural selection. In his epoch-making book *The Origin of Species*, Darwin expresses his approval of Sprengel's conclusions and reports additional experiments confirming the role of insects in pollination.[22] 'Poor old Sprengel', Darwin noted after publishing the *Origin*, 'his merits have only now, so many years after his death, been fully recognised.' Recognized by him—Darwin—in modest wording.

In marked contrast to the animal world, the majority (about 80%) of angiosperm plants are bisexual. They bear hermaphrodite flowers, that is, they possess both stamens and a pistil. Only about 10% of the world's flora have staminate and pistillate flowers on separate plants (dioecy). The principle of bisexual flowers could easily promote high levels of self-fertilization and consequently inbreeding, but, as indicated above and as noted by Sprengel and Darwin, plants avoid self-fertilization. As this feature is of crucial importance in the evolution of plants, various mechanisms have been developed to prevent self-pollination. They are of two sorts: those that separate the sexes either in time or in space (for instance by differences in the timing of maturation of stamens and ovaries) and, second, self-incompatibility mechanisms. The latter method is based on a plant's ability to discriminate between its own pollen grains and those of another plant, and to allow only pollen from a different plant to grow pollen tubes and fertilize the ovules.

Approximately two-thirds of all flowering plants are pollinated by insects. This service is not given *gratis*. In return for pollen transfer, plants provide food to their pollinators in the form of nectar and pollen. These are desirable nutriments: nectar may contain 50% sugars and pollen 15–60% proteins and other essential elements.[96,106] Because the two parties can survive barely or not at all without each other, this is an exemplary case of *mutualism*. Associations from which both partners benefit are widespread, but that between angiosperms and insect pollinators is probably the most spectacular and large-scale example of mutualism in the living world.

Although insects and flowers form an example *par excellence* of mutualism as a principle, the degree of mutualism varies among species, and the degree of interdependence of flowers and pollinators covers a broad spectrum. At one end of the spectrum the partners are highly specialized and the interaction is a question of life and death. Figs, for instance, can be pollinated only by specialized fig wasps—a specialization that reaches the extreme, because each fig species is pollinated by its own species of wasp. Female wasps pollinate and lay eggs within the flowers. The offspring develop within the seeds, eclose as adults and, still inside the fruit, mate. The females then fly off to lay their eggs in another fig inflorescence, which may be located over distances of 10 km or more. The migrating female serves as a pollen transport vehicle. After oviposition, she dies within that fig.[14,85] Here the interdependence is absolute.

At the other end of the spectrum the relationship is antagonistic.[123] For example, *Ophrys* orchids can be regarded as 'sexual parasites' of their pollinators (see below), which can do very well without these flowers. Other insect–flower relationships are situated somewhere between these two extremes.

12.1 Mutualism

Many plant species that have conspicuous, coloured, and scented flowers require insect pollination to optimize seed production (Table 12.1).[10] Birdsfoot trefoil (*Lotus corniculatus*), for instance, produces practically no seeds in the absence of pollinators. Just one single honeybee visit results in the production of several seeds per flower, but to achieve maximum pollination as many as 12 to 25 visits are required.[84] The flowers of yellowbog saxifrage (*Saxifraga hirculus*) also must be visited many times to ensure optimal seed-setting. After about 200 visits of pollinators, during which roughly 350 pollen grains are deposited on their stigmas, these flowers produce an average of 30 seeds per flower.[91] Thus, flowers usually have to be visited more than once to maximize and to optimize seed-setting. Different visitors bring pollen from different fathers and the risks of pollinators bringing incompatible pollen are compensated. In agricultural and horticultural crops fertilization

Table 12.1 Effects of excluding insect visitors (primarily bumblebees) on the seed production of four ericaceous plant species in a bog ecosystem. The percentage of fruits producing seeds was compared for shoots enclosed in mesh bags and unenclosed shoots (modified from Reader, 1975)[100]

Plant species	Seed production (%)	
	Enclosed	Unenclosed
Wild rosemary (*Andromeda glaucophylla*)	0.7	33.6
Swamp laurel (*Kalmia polifolia*)	0	55.6
Labrador tea (*Ledum groenlandicum*)	1.0	96.2
Large cranberry (*Vaccinium macrocarpon*)	4.0	55.7

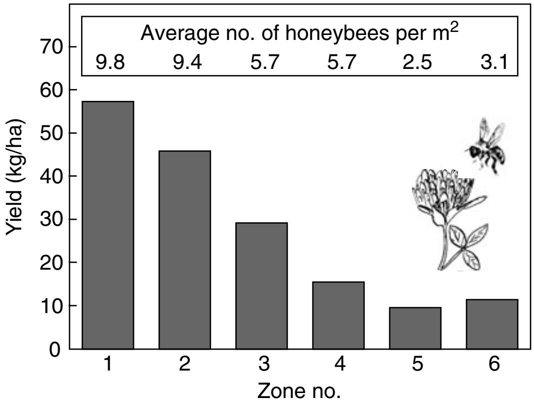


Figure 12.2 Seed yields of red clover (*Trifolium pratense*) decrease as distance from honeybee colonies on the edge of the field increases. Seed production was measured in six zones, each 122 m wide and parallel to the field edge. Zone 1: 0–122 m from the bee hives; zone 6: 610–732 m. Figures represent number of honeybees observed in the various zones per unit time. (Data from Braun *et al.*, 1953.)[9]

and seed production is often suboptimal because there are insufficient numbers of natural pollinators. In that case yields can be improved considerably by moving honeybee colonies into the crop area (Fig. 12.2).[30]

From the insects' point of view, pollen and nectar constitute important food sources. Apoidea (bees and bumblebees) even receive all of their nourishment from these two flower products and they are well equipped to collect relatively large quantities of them. Bees are covered with a dense coat of feathery hairs that, through the presence of small hooks, effectively catch and hold pollen grains when the insect touches the anthers of a flower. The stickiness of pollen from insect-pollinated flowers facilitates their adherence to an insect vector (Fig. 12.3). Pollen from wind-pollinated plant species lack the oily 'pollenkit' cover and is not sticky. In addition to its pollen-carrying capacity the

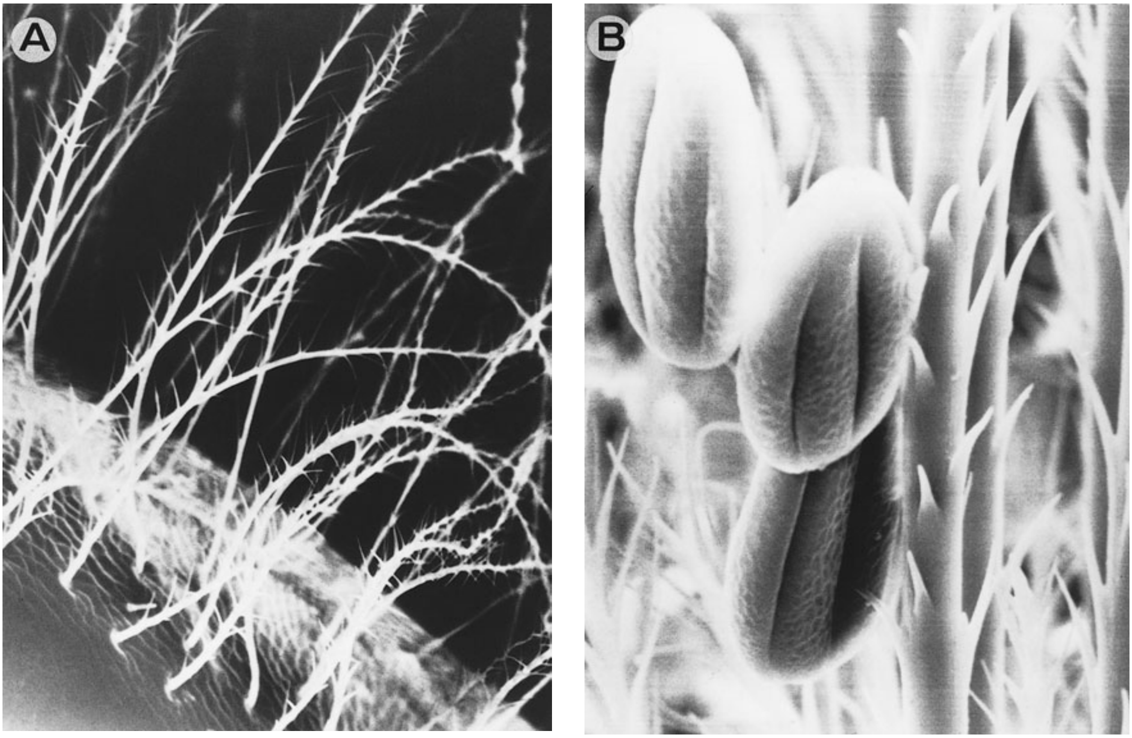


Figure 12.3 (**A**) Plumose hairs covering the body surface of honeybees (*Apis mellifera*) have teeths and hooks, which assist in collecting pollen. Original magnification × 160. (**B**) Hairy coat of bumblebee head with some pollen grains sticking to the spiny hairs. Original magnification × 725. (From Barth, 1985.[5] © 1985 by Princeton University Press.)

insulating fur allows its owner to maintain high body temperatures and thus to be active at low air temperatures.[47] During flight the bee rakes with its legs all the pollen from its hairy body surface and collects it in pollen baskets situated on the outer side of the tibia of both hind legs (Fig. 12.4).[114]

With this device a honeybee worker may carry a pollen load of as much as 10–20 mg home to the hive. A honeybee colony, consisting of approximately 10 000 to 50 000 insects, consumes approximately 20 kg of pollen and 60 kg of honey per year. The pollen provides the protein for growth and reproduction. To rear one honeybee about 125 mg is required, an amount equalling the bodyweight of the adult.[113]

Nectar, which in composition bears some resemblance to phloem sap, contains anything from 10% to 70% sugars by weight. A wide range of other compounds, such as free amino acids, lipids, minerals, and secondary compounds, are also present, albeit in small quantities. Whereas sugars are a most valuable reward because of their energy content, the occurrence of amino acids in nectars is attractive to those pollinators that lack alternative resources.[2]

Strangely enough, more than a few plant species produce nectar that is toxic or repellent to some floral visitors. Several hypotheses have been proposed to explain this seemingly anomalous feature of nectar, but as yet no satisfactory explanation based on hard experimental evidence is available.[1]

Although plant and pollinator fully depend on one another, there is at the same time—as in any mutualism—an intrinsic conflict between the parties, in that each is under selection for increased exploitation of the other. Plants need to receive as many conspecific pollen grains on their stigmas as possible and the reciprocal transfer of their pollen to flower stigmas of other conspecifics. The ideal vector would, during each visit, contact anthers and stigmas, move rapidly among plants and search exclusively for conspecific flowers, even when other flowering plants abound. In order to force its pollinator to visit many flowers, selection favours the secretion of a sufficient amount of nectar to be attractive to bees and reward them for taking the trouble to make the visit, but not so much that pollinators need to visit only a few flowers per trip to imbibe a full nectar load and go home. From the plant's perspective a harried, hungry, and yet plant-species-constant pollinator is ideal. Insects, on the other hand, according to optimal foraging theory, will try to collect as much food as possible while minimizing energy and time expenditure. This means that flowers with copious nectar flow will be preferred and that it may be more efficient to visit flowers of different species during a foraging trip. This conflict between the

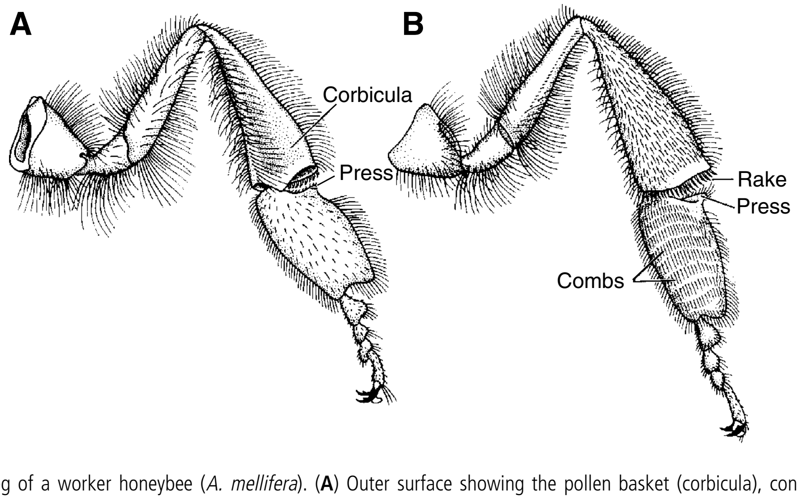


Figure 12.4 Hindleg of a worker honeybee (*A. mellifera*). (**A**) Outer surface showing the pollen basket (corbicula), consisting of a bare concavity fringed with stiff hairs. The 'press' forces the pollen into the basket. (**B**) Inner surface with 'combs' and 'rakes', which manipulate pollen into the press before it is pushed into the lower end of the basket. (From Snodgrass, 1956.)[114]

interests of plants and their pollinators must have been a major force in shaping present-day plant–pollinator relationships.[28,62]

12.2 Flower constancy

Individual honeybees often restrict their visits to flowers of a single species and ignore many other suitable and rewarding flowers of alternative plant species that they pass on a foraging trip. The tendency to specialize has been referred to as *flower constancy*. The phenomenon of flower constancy is of crucial importance to pollination ecology and evolution, and thus deserves special attention.

Pollinator fidelity not only improves foraging efficiency, but also helps the reproductive isolation of plant species, and thereby the maintenance of species differences. This type of specialization implicates learning processes based on flower recognition from a distance and on acquiring the skill to collect pollen and nectar from flowers of different architecture. The advantage of flower constancy behaviour has been ascribed to a limited ability to learn or to remember how to deal simultaneously with many different flower types.[132] Accordingly, a bee would forage more efficiently if its sensory system and behaviour were temporarily fixed in a particular way. To realize this the insect seems to use a perceptual mechanism akin to a 'search image' to find flowers, just as herbivores do during food-plant selection and as predators do in terms of their prey-selection patterns. Experimental support for this assumption is, however, still very limited.[34,38]

Flower constancy can be measured by examining the composition of loads of pollen on the basis of their characteristically marked walls, which are typically sculptured, punctured, crossed with bonds, spined, or recognizable by other features of the grain exine (Fig. 12.5).[83] Flower constancy is usually expressed as the percentage of individuals with pure loads at the end of a foraging trip.[21,86] Analyses of pollen load composition have revealed that many bee species show high degrees of flower

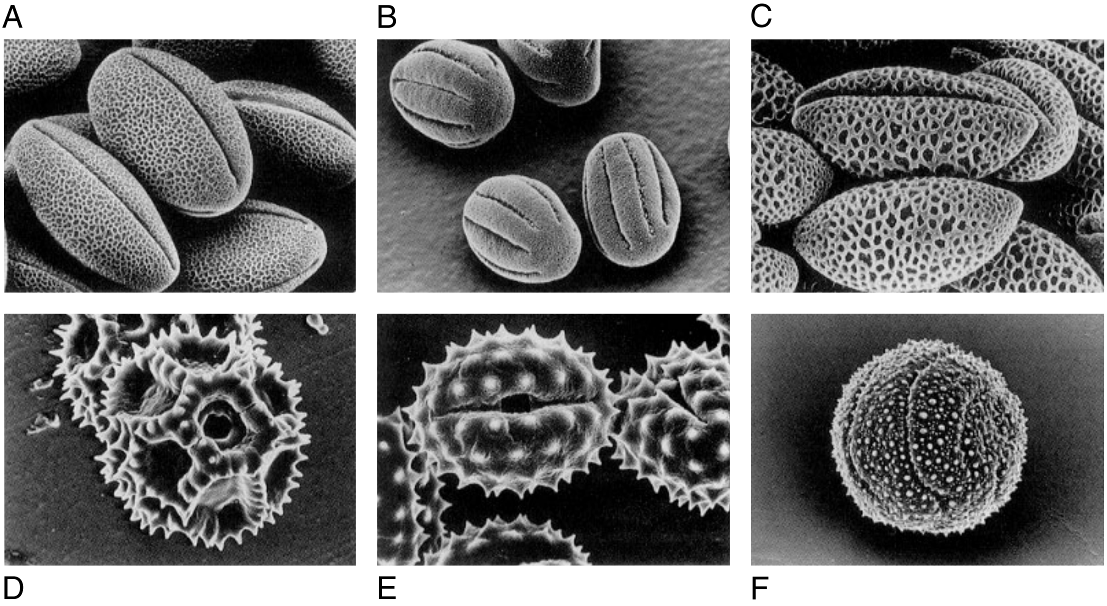


Figure 12.5 Pollen grains. (**A**) Black horebound (*Ballota nigra*); three longitudinal furrows. (**B**) Marsh bedstraw (*Galium palustre*); grain with many longitudinal furrows. (**C**) Lesser hawkbit (*Leontodon saxatilis*). (**D**) Ragwort (*Senecio jacobaea*). (**E**) Flowering rush (*Butomus umbellatus*). (**F**) Pipewort (*Eriocaulon aquaticum*); the furrow follows a spiral, like peeling an orange. Scanning electron micrographs of air-dry pollen, × 870. (From Proctor *et al.*, 1996.)[96]

constancy, as shown in Table 12.2. They are even more constant than the figures in this table suggest, because the definition of a pure load is a strict one. 'Mixed' pollen loads often contain only very small amounts of pollen from one or more other plant species. For instance, of the 19% 'mixed loads' of honeybees in Table 12.2, every one was 95–99% pure.[43]

The duration of a period of flower constancy may vary considerably. Often bees keep to one flower species only during a single trip. Other individuals show longer periods of fidelity and visit the same kind of flower for several hours or days. Different workers of a honeybee colony may show constancy to different flower species, and different colonies as a whole may also be specialized on different flowers. However, absolute flower constancy would be counterproductive and prevent insects from discovering more rewarding resources. Thus, solitary bees constantly check other flower species to assess whether more rewarding species are available, and consequently show lower degrees of flower constancy than, for example, honeybees. In the latter case efficiency is increased by 'scouts', which constantly monitor, sample, and pool information about the best food sources available and, by employing their highly developed communication system, 'instruct' the 'recruits' on which sources to visit.

From both pollen load analysis and direct observations in the field it appears that not all kinds of pollinator show the same degree of faithfulness to one flower species. Although social bees are superior to other groups, a tendency to visit successive flowers of the same species has also been observed outside the Apoidea, for instance in some butterflies, and to a lesser extent hoverflies (Syrphidae).[41,74,134]

Table 12.2 Flower constancy of some Apoidea (from Grant, 1950)[43]

Genus	Pure pollen load (%)
Apis (honeybee)	81
Megachile (leafcutter bee, solitary)	65
Bombus (bumblebee)	55
Halictus (sweet bee, solitary)	81
Andrena (mining bee, solitary)	54
Anthophora (mining bee, solitary)	20

Flower constancy is expressed as the percentage of individuals with pollen from one plant species only. The figures do not include the (many) individuals that carry only a small fraction of different pollen species.

12.2.1 Flower recognition

A high degree of flower constancy requires not only the ability to learn quickly to recognize a rewarding flower species but also the capacity to identify conspecific flowers rapidly on the basis of characteristics sufficiently specific to minimize the chance of error. Many pollinators can rapidly associate several flower characteristics (e.g. shape, colour, and odour) with food reward.

Among the visual cues, colour is one of the most important signals by which a pollinator locates, recognizes, and discriminates between flowers at some distance. Memorizing the features, such as colour, of rewarding food sources increases, of course, the efficiency of foraging behaviour. Honeybees are able to remember a colour reliably (i.e. with an accuracy of 90%) after about six rewarding visits (Fig. 12.6). Cabbage butterflies (*Pieris rapae*) do even better: they can select the colour on which they have been fed only once with about 82% accuracy.[75] Experiments with another butterfly, the

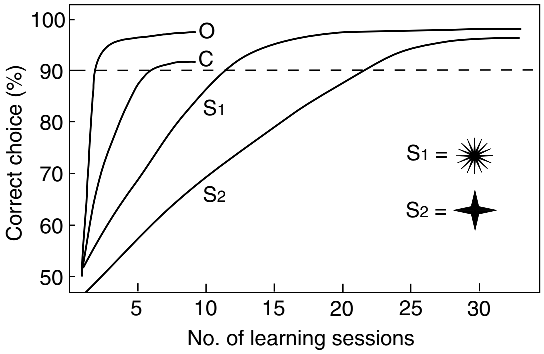


Figure 12.6 Learning curves for odour (O), colour (C), and shapes (S) in honeybees show that a typical floral scent is learned more rapidly than an average colour, and that the accuracy of odour memory is higher. Shape learning appears to be more difficult than either odour or colour learning, although it eventually reaches a roughly equivalent level of accuracy. Highly subdivided shapes, such as the 16-pointed star (S1), are learned faster than simple figures, such as the four-pointed star (S2). (Redrawn from Menzel *et al.*, 1974,[80] and Schnetter, 1972.[112])

papilionid *Battus philenor*, revealed that the colour-learning capacity of butterflies is still more impressive because it shows concurrent associative learning of two different stimuli in two different behavioural contexts: food foraging and oviposition behaviour. Such dual conditioning permits female butterflies to forage effectively for nectar resources and egg-laying sites even when those activities are intermingled in time.[136]

The selective spectral reflection of flowers and the colour vision systems of pollinators have over the course of time developed together in a mutual relationship. It is a long-held misconception that insects are unable to perceive red flowers, as red is included in their colour world.[13]

To exploit the flower constancy of Hymenoptera optimally and to prevent 'mistakes' by its pollinators, a plant should have floral colours as different as possible from sympatric heterospecific flowers. Flower colours of different angiosperms do show sharp steps in their spectra at precisely those wavelengths at which the pollinators are most sensitive to spectral differences.[12] Examination of the whole flora of particular habitats has shown that the colours of the flowers are more diverse and more discrete to insects than to humans. These findings indicate an evolutionary tuning of flower colours to the sensory system of bee pollinators, or, alternatively, the result of a co-evolutionary process.[79]

Shapes and patterns appear to be more difficult to learn, and 10 to 30 trials are required to reach approximately the level of accuracy equivalent to that of colour memory (Fig. 12.6). Interestingly, more complex shapes can be learned faster than simple ones, owing to an innate preference for shapes with high figure intensities, that is, figures with a high ratio between the contour length and the area enclosed. Attractiveness to visual cues can be further increased by the presence of nectar guides, adding once more to a flower's visual complexity.

A particular aspect of flower shape is the perfection of its symmetry, whether it be radial or bilateral. The high accuracy of form perception in bees (and other insects) is accentuated by the finding that they are able to detect symmetry imperfections. As perfectly symmetrical flowers seem to produce more nectar than flowers with high levels of fluctuating asymmetry, pollinators exert selection pressure on flowers for shape regularity.[36]

A high degree of flower constancy requires not only the ability quickly to learn a rewarding flower species but also the capacity to identify conspecific flowers rapidly on the basis of characteristics sufficiently specific to minimize the chance of error. The rich menu of volatiles produced by flowers plays a prominent role in the phenomenon of flower constancy. Correspondingly, olfactory discrimination in pollinators is highly developed and, likewise, their learning capacities are often impressive. Honeybees can learn a floral odour with a reliability of 93–100% after only a single exposure, whereas conditioning to colours, as we have seen, takes four to six visits (Fig. 12.6). Single odorous compounds, interestingly, are usually more difficult to remember than composite floral scents (Fig. 12.7). Combinations of odours and colours are even more easily remembered after offering them together with a food reward.

The fragrance signature of flowers is a composite of volatile chemicals in specific stochiometric concentrations.[98] Typically, these compounds are monoterpenoids and sesquiterpenoids, benzenoids, phenylpropanoids, and fatty acid derivatives. Despite their great diversity, these compounds are

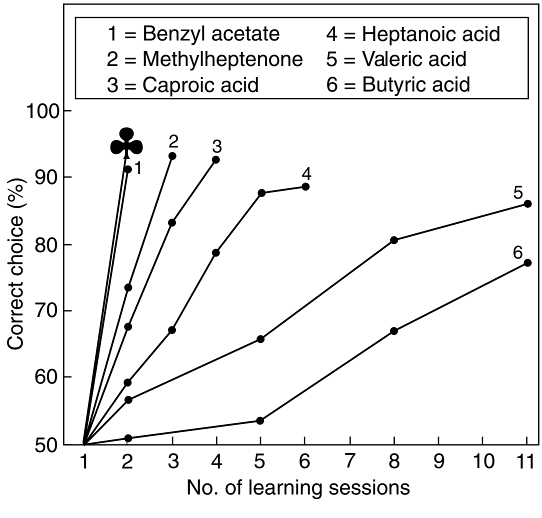


Figure 12.7 Odour learning curves in honeybees for a flower fragrance (fennel = ♣) and various pure chemicals. (From Kriston, 1971.)[67]

biosynthesized by a relatively small number of (often overlapping) metabolic pathways.[126]

Most floral volatiles are bouquets of at least a few but usually many components. Although the blend is often dominated by one or a few main components,[24,56,64] this does not necessarily mean that these form the most important signal to the insect. Thus, chromatographic analysis showed the scent of sunflowers to be a mixture of as many as 144 constituents. No fewer than 28 of them are relevant for constituting 'sunflower odour' as perceived by honeybees, indicating a finely tuned olfactory system in these highly adapted insects.[94] The major part of the bees' behavioural response to an odorous mixture, however, is elicited by only a small number of key compounds.[69]

Given the large number of possible combinations of flower odour components and the fact that bees show the capacity to discriminate thousands of odour mixtures, they must be able to recognize many flowers by their scents alone. Experimental evidence has shown that honeybees can indeed learn and distinguish at least 700 different floral aromas. Floral scents, however, not only serve as identifiers, assisting the harried bee to recognize the flower species, but also enable a pollinator after landing to forage efficiently. For that reason flowers may show spatial patterning of fragrance emission within a flower, in both the kind and the amount of volatiles produced, forming an odorous nectar guide (Fig. 12.8). Such an odour trail, in concert with tactile and gustatory stimuli, helps an experienced insect rapidly to find the pollen or nectaries.[24,25] Nectar may also contain odorous compounds that could serve as a sensory cue to beneficial and/or harmful floral visitors. Some instances are known of nectars containing volatiles that differ from scent compounds of other floral tissues. Conceivably such differences may facilitate their rapid detection by pollinators and, as a consequence, reduce flower-handling time.[97]

The observation that pollen produces characteristic volatiles, often quite different from the overall scent of a flower, is seen as evidence that pollen is deliberately provided as a reward to insect pollinators. This assumption is supported by the fact that bees can discriminate between plant species on the basis of pollen odour.

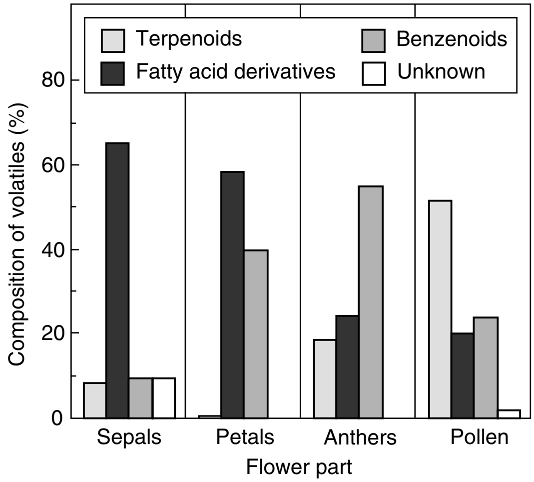


Figure 12.8 Percentage of four classes of volatile compound produced by different parts of the flowers of *Rosa rugosa*. (Data from Dobson *et al.*, 1990.)[25]

The overall taste of nectar is possibly determined by its amino acid content. Different nectars show great variation in amino acid profiles that may be relevant taste cues for pollinating insects. Foraging choices by adult butterflies for which nectar is the only source of nitrogen are affected by amino acid concentration, and honeybees respond to amino acid variations in nectar mimic solutions. Thus, different suits of amino acids, as found in different nectars, could very well contribute to flower recognition by pollinators and consequently also play a role in flower constancy behaviour.[33,81]

Flowers are, in addition to colour, shape, and smell, characterized by the microtexture of their petals. Their surfaces are covered with many kinds of microscopic ridges, pimples, and plates, which could serve as recognition cues for pollinators. Bees can use such texture characteristics to discriminate between upper and lower petal surfaces and the back and front of a flower. Apparently touch is another, possibly important, sensory channel in the complex process of flower recognition.[60]

12.2.2 Flower handling

After landing on a flower, naive bees show an innate probing response, but they must learn how to exploit flowers of increased complexity efficiently. As learning involves the investment of time

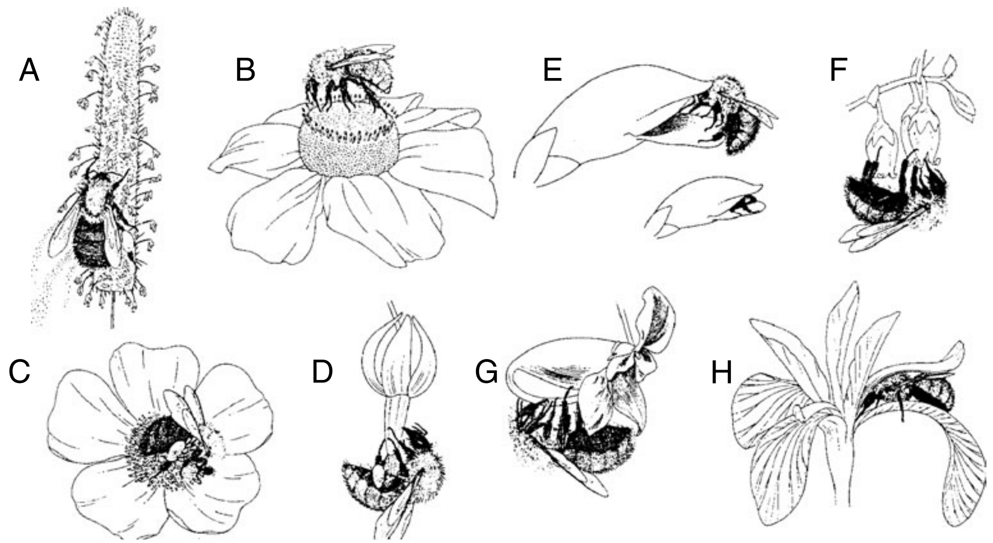


Figure 12.9 Handling of different kinds of flower by bumblebees collecting nectar or pollen. (**A**) Walking up inflorescence of grass (*Phleum* sp.) collecting pollen. (**B**) Collecting nectar and possibly pollen from a composite flower. (**C**) Grasping and vibrating groups of anthers in *Rosa* sp. during pollen collecting. (**D**) Holding *Solanum dulcamara* blossom with legs and mandibles while shaking pollen from the tubular anthers by vibrating the flower. (**E**) Entering *Chelone* blossoms. (**F**) Collecting nectar from *Vaccinium* blossoms. (**G**) 'Robbing' nectar via a whole bitten in the spur of *Impatiens* sp. (**H**) Iris blossom being visited for nectar. (From Heinrich 1976, with permission.)[45]

and energy, it befits a bee, once a flower species has been successfully probed, to continue to forage from it. Learning how to manipulate complex flower types is no easy task.[70] Different types of flower, with their nectaries often hidden in very specific places, require different handling techniques, and bees have to learn such things as where exactly to alight, where exactly the nectaries are located, and how to reach them as quickly as possible (Fig. 12.9).

Food-finding is relatively simple on the flat-topped inflorescences of Apiaceae where bees, while rapidly moving around, collect pollen from the tiny flowers by pressing their bodies to the suface. More advanced procedures are needed on more complex flowers, such as *Chelone glabra* (Scrophulariaceae), where the petals must be pried apart to obtain access to the nectar, or monkshood (*Aconitum* spp.) flowers, where the bee, after entering at the bottom, must pass over the anthers to reach the nectaries concealed deeply inside (Fig. 12.10).

Flower-handling techniques learned on one plant species presumably interfere with previously learned techniques for other plant species because of limited neural capacity. Bumblebees with no experience in handling flowers with complex morphology and limited access to the nectar, such as jewelweed (*Impatiens biflora*), often could not find

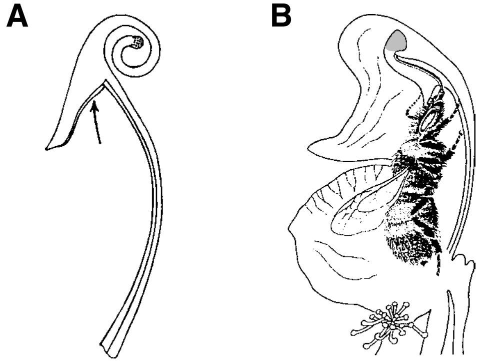


Figure 12.10 Monkshood flowers contain two vertical nectar petals, partly shaped into a tube, with nectaries located at the very end. (**A**) Nectar petal of *Aconitum vulparia* (arrow indicates tube entrance). (**B**) Flower of *Aconitum variegatum*, with worker bumblebee inserting its tongue into nectar petal. The bee, after entering at the bottom, must pass over the anthers to be able to probe into the tips of the two nectar petals. Monkshood species occur only in parts of the world where bumblebees occur. ((**A**) from Knoll, 1956,[63] and (**B**) from Laverty and Plowright, 1988.[71])

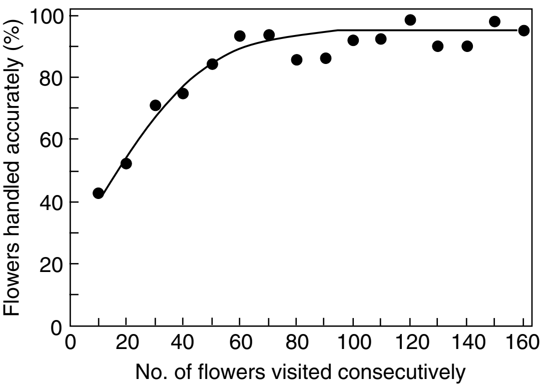


Figure 12.11 Improvement in handling success of naive bumblebees after 1 to 160 contacts with *Impatiens biflora* flowers. (Redrawn from Heinrich, 1979a.)[46]

the rich nectar content. It took about 60 to 100 flower encounters before they had fully developed the skill to extract the nectar (Fig. 12.11).[46] The strategy of flower constancy must surely increase foraging efficiency, because the insect, once it knows where the nectaries of a particular type of flower are located and how to reach them with the least effort, certainly saves energy and time.

12.3 Pollination energetics

Mutualistic relationships between two groups of organism benefit both partners, as manifested by increased fitness. According to optimization theory, organisms try to maximize their survival chances and reproductive success by balancing costs against benefits for each activity or function. The application of cost–benefit analyses to insect–flower mutualism has proved extremely useful in understanding the degree of mutual dependency. Optimal foraging theory holds that foraging strategies involve decisions that maximize the net rate of food intake (i.e. net caloric gain per unit of time).

Pollination energetics can be studied in two ways. The first is a conceptual analysis based on models that account for the behavioural and physiological mechanisms that underlie foraging. The second approach aims to test in the field the predictions of the models on the energy balance of foragers. Factors that may be relevant for a cost–benefit analysis of a foraging insect are manifold, but include as a minimum the distance to the food source, its accessibility, the amount and quality of the food, and the ambient temperature. These four basic elements are discussed below.

12.3.1 Distance

Honeybee foraging normally extends over a vast area around a colony's nest. A detailed study of one colony in a deciduous forest showed that the most common forage patch distance was 600–800 m, but many individuals flew out several kilometres from the nest. Because 95% of the colony's foraging activity occurred within a radius of 6 km, the food source area of this colony could be set at more than 100 km^2.[113] Depending on foraging habitat and other environmental conditions, foraging ranges are sometimes even considerably greater.[7] Bumblebees, likewise, often forage at distances of several hundred metres or even kilometres from their nests.[131]

Food collection requires an enormous expenditure of energy. A foraging bumblebee weighing 500 mg spends as much as 600 J per hour, which is equivalent to the energy bound in 40 mg of glucose. Flight activity accounts for by far the greatest share of energy consumption. Hovering in front of a flower, as larger insects often do to extract nectar, is particularly costly. To economize on energy expenditure bees will travel to distant food sources only if the reward makes the trip profitable. The higher the sugar concentration at an experimental feeding station, the further honeybees will forage on it. The decision to collect food at a distant source takes into account not only the energy required to get there by flight, but also loss of travel time. Therefore, the relationship between distance and minimum food concentration to make the trip worthwhile is not linear but takes an exponential form (Fig. 12.12). Flowers at 3 km from the colony should provide at least 3.4 times more nectar than flowers near the hive to make foraging on them attractive. In spite of an appreciable energy consumption, bees are highly efficient flyers.[138] The travel costs of a return flight to a food source located 4.5 km from a bee's nest are the equivalent of only 10% of the nectar yield.

In nature, bees are confronted with variability in nectar content among flowers. Do they notice it and, if so, does it bother them? In an elegant experiment by Real and co-workers, insects were allowed to forage on a patch of artificial flowers of two distinct colours. All blue-coloured flowers contained the same reward. The yellow-coloured flowers contained variable amounts of sugar water, but on average had the same amount as the blue ones. Bumblebees as well as paper wasps preferred the blue flowers with the lower variance in reward. However, when the mean sugar content of the high-variance flowers was raised, the insects preferred to forage on this more risky type. Apparently the bees' foraging strategy included a certain degree of risk avoidance, which could be offset by increased gain.[101] Thus, distance is one parameter on which foraging decisions are based; predictability is another, and the caloric worth of nectar rewards is a third.

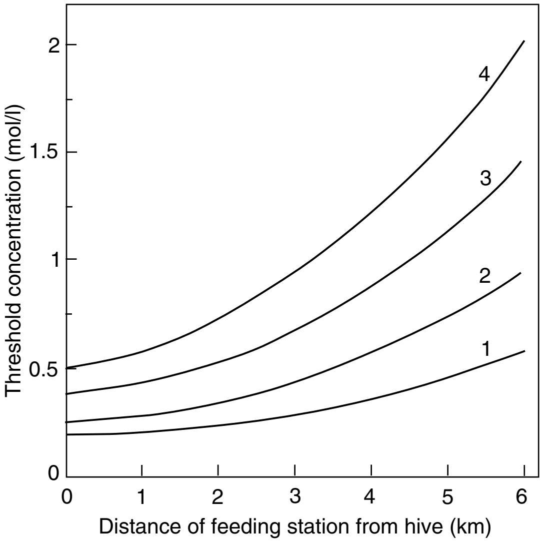


Figure 12.12 Honeybees finding a sugar solution recruit other bees in the hive only when the feeding station presents a sugar solution above a threshold concentration. The threshold increases with distance. Threshold concentrations vary with environmental variables, such as the presence of alternative food sources and weather conditions. The responses on four different days (1–4) are represented by four different curves, reflecting different environmental conditions. (Data from Boch, 1956.)[8]

12.3.2 Accessibility

Flower morphology affects the time needed to find and collect the nectar or pollen. Shallow, open-cup flowers require little handling skill, as the nectar is accessible from any position on the flower. Complex flowers, such as monkshood (*Aconitum* spp.; see Fig. 12.10), demand more complex handling methods from their visitors because locating the reward is more difficult. It also takes more time. The flower compensates the pollinator for increased investment in time and effort by providing a rich nectar reward.

The number of trials and the time needed for naive bumblebees to learn flower handling increases with floral complexity (Table 12.3).[70] The combination of copious rewards with a floral morphology requiring high learning capabilities of its pollinators promotes flower constancy.

12.3.3 Temperature

Bumblebees can be seen collecting food at temperatures near freezing or, in the Arctic, even below 0°C. Honeybees become active, depending on the

Table 12.3 Flower-handling time and foraging success of bumblebee workers on nine plant species with different floral complexity (modified from Laverty, 1994)[70]

Flower type and plant species	Handling time (s)		Foraging success (%)
	1	55	1
Open-cup flowers			
Apocynum sibiricum	5.5	0.4	100
A. androsaemifolium	14.1	0.3	100
Open-tube flowers			
Prunella vulgaris	13.9	0.1	100
Vicia cracca	18.1	0.2	100
Impatiens capensis	20.4	1.7	70
Closed-tube flowers			
Gentiana andrewsii	44.4	6.5	45
Chelone glabra	196.6	8.1	40
Monkshood flowers			
Aconitum henryi	134.7	13.6	35
A. napellus	153.5	3	29

Figures are given for bees visiting a flower species for the first time (1) or after 54 earlier visits (55) to the flower species.

season, between 10 and 16°C. Bees can forage at cool temperatures because they are *endothermic* and fly with a minimum thorax temperature of 30°C. They maintain a high body temperature by the heat produced from their flight metabolism and, when not in flight, by shivering their flight muscles with the wings uncoupled.[47,130] Foraging at low temperatures is, however, expensive energetically. Food-collecting bumblebees at 5°C spend two or three times more energy than at 26°C to keep their thorax temperature at 30°C or higher (Fig. 12.13). An increased temperature is a prerequisite for normal functioning of the flight muscles. To maintain high body temperatures, bees possess an unusually high activity of the enzyme fructose-1,6-diphosphatase, which enables heat generation by ATP hydrolysis. In some bumblebees the activity of this enzyme is up to 40 times that in the honeybee, allowing them to forage at substantially lower temperatures than honeybees.

Investment in extra heat production under cold weather conditions must of course be compensated by high energy intake. Bumblebees can therefore be seen to forage in cool weather on rhododendron blossoms, which yield profitable amounts of nectar, while neglecting wild cherry (*Prunus avium*) and lambkill (*Kalmia angustifolia*) blooms, because these flowers produce too little nectar to break even in energy terms under these conditions.

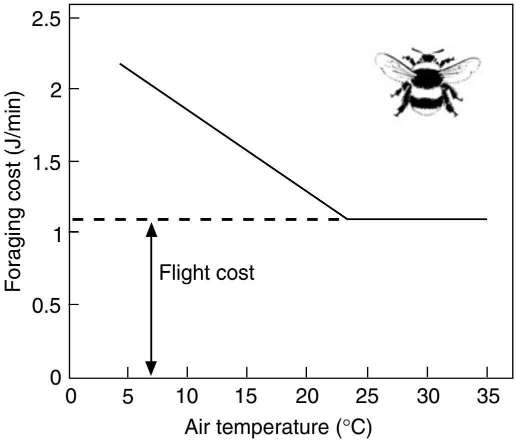


Figure 12.13 Calculated costs in relation to air temperatures for a bumblebee that regulates its thorax temperature at 30°C and spends half its time in flight and half on handling flowers. All costs above the dashed line are contributed to thermoregulation. (From Heinrich, 1979b.)[47]

Once the energy requirements of a forager at different temperatures are known, as well as the fuel needed to fly per unit of time, the extractable energy content per flower, and the average distance between flowers, predictions can be made as to which flower species will be visited at various temperatures and which will not. Heinrich, in a fascinating book entitled *Bumblebee Economics*, has shown on the basis of ingenious experiments that bumblebees employ a thermal strategy that accounts for many variables and thereby ensures maintenance of a positive energy balance.[47]

Flowers that open early in the morning are visited mainly by large insects, which can regulate their body temperature. As temperatures rise, small pollinators become active. In the early morning the blossoms of *Arctostaphylos otayensis* (Ericaceae) were found each to contain 6.3 J of sugar. When foraging at 2°C, bumblebees need about 3.4 J/min, in order to make an energy gain on these flowers, even while foraging at near-frosty temperatures. At noon each flower contained only 1.3 J of sugar. By that time bumblebees had lost interest and the flowers were visited predominantly by small insect species.[48]

To make flower visitation profitable in cool weather conditions, nectar production should be relatively profuse or the flowers should grow closer together than under high temperature conditions so that they can be visited in rapid succession. The tendency for spring flowers to grow in clumps may be a strategy on the part of the plant relevant to pollination success. In addition, the fact that plants growing further north secrete more nectar than conspecifics at lower latitudes, and similar trends on elevation gradients, suggests an adaptation to the greater energy needs of their pollinators.[48]

Although our knowledge of the relationship between nectar provision and the energy requirements of pollinators is far from complete, there is sufficient evidence to conclude that supply and demand are finely tuned to each other.

12.3.4 Food-source evaluation

Honeybees returning from a foraging trip communicate details about location and quality of the food source to other members of the colony by means of the famous 'bee dance'. Detailed analysis of this ritualized act and correlation of its subtle modifications with various manipulations of food sources allowed Karl von Frisch and co-workers to determine which factors the bees use to calculate the profitability of their foraging activity.[32] They found that a whole range of food-source characteristics are taken into account in the nature and duration of the dance, such as distance, nectar quality (i.e. sugar concentration), viscosity, ease of obtaining the nectar, uniformity of flow, odour, time of day, and weather conditions. In addition to the direct costs (i.e. flight and handling energy, and time), bees making foraging decisions probably also 'reckon' indirect costs such as risk of predation and body wear and tear.[50] It is unclear how time, for instance handling time, is measured, but some evidence suggests that when measuring foraging gains and costs bees integrate their time budget in some way or other into their energy budget, and hence estimate time in terms of energy units.[129]

Thus, studies on pollination energetics include factors such as:

(1) pollinator foraging behaviour (distance to foraging area, interplant flight distance, departure decisions, movement patterns, speed);
(2) reward type and quantity (pollen, nectar composition, caloric value, and spatiotemporal distribution);
(3) flower-handling costs (pollinator energy and time expenditure).

12.3.5 Reward strategy

To promote outcrossing, plants need visitors, such as insects and some vertebrates, which are rewarded for their service. Flowers must provide sufficient nectar to attract foragers, but they must limit this reward so that pollinators will go on to visit other plants of the same species.[62] Nectar secretion per flower or per plant is carefully optimized and adapted to time of day (Fig. 12.14), season, and the kind of pollinators the plant prefers to employ. A 100-mg bumblebee may expend about 0.3 J/min after landing on a flower, whereas a 3-g sphinx moth imbibing nectar while hovering at the entrance of a flower expends about 140 times as much.[48]

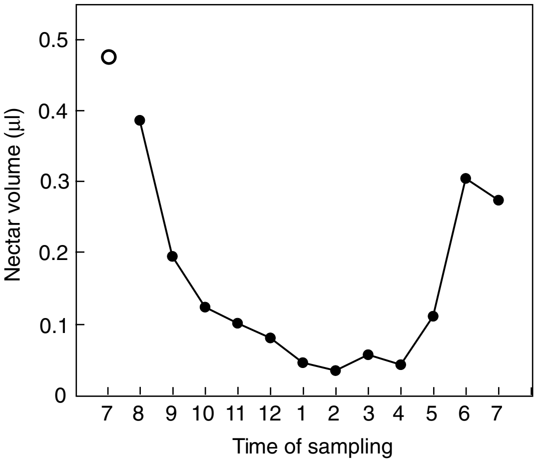


Figure 12.14 Mean volumes of nectar secreted per hour by *Cerinthe major* blossoms during 1 day in the absence of insects. The first reading (open circle, at 07:00 hours) represents the overnight accumulation of nectar. (From Gilbert *et al.*, 1991.)[35]

Nectar production obviously exerts a drain on the plant in terms of carbon demand, but as yet it is difficult to quantify production costs in terms of growth and/or reproduction. All we can say at present is that the energy spent on nectar secretion varies greatly between plant species.[23] Whatever the level of energy investment, it seems logical to presume that plants try to reduce the losses by limiting nectar flow as much as possible and to direct the energy saved to seed production. This has led to the supposition that some plant individuals may attempt to cheat on their conspecifics and save energy by secreting little or no nectar at all. Bees that are conditioned to a particular flower species will continue to visit many more without receiving a single reward. This kind of deception is an example of *automimicry*.[20] The interactions between bees, nectar-producing plants, and cheating automimics can be understood in more formal terms by employing game theory,[78] as has been done in a study of the nectar flow of individual flowers of *Cerinthe major* (Boraginaceae). About 25% of these flowers produced copious amounts but the remaining flowers secreted only small quantities. The observed ratio between high secretors and low secretors closely fitted the value predicted on the basis of flower type, amounts of nectar produced, and mean discrimination and handling times for a particular forager.[35]

Other reports of substantial differences in nectar productivity between plants have also indicated that plants can employ various strategies and vary the proportion of cheating (i.e. nectarless) flowers. An investigation of 28 species of plants revealed that 24 of them belonging to 23 genera and 16 families had nectarless flowers. Among those species, the frequency of nectarless flowers on an individual plant was on average 24%. The commonness of this phenomenon suggests that the presence of some or even many nectarless flowers increases the fitness of a plant. The fact that nectarless flowers do not have to pay the cost of nectar production, together with the (presumed) incapability of pollinators to discriminate between nectarful and nectarless flowers, supports the hypothesis that plants can reach an evolutionary stable proportion of 'cheating' flowers.[121] Clearly there is room to cheat your pollinators.

Amino acids are found in floral nectars of primitive angiosperms, albeit in relatively small amounts. In some other plant taxa they occur in significant quantities. Their concentrations vary from 0.4 to 4.7 mM in herbaceous species. The discovery of a correlation between between pollinator type and amino acid concentration in nectars led to the idea that their presence reflects an adaptation to pollinators that have no alternative nitrogen resources, such as butterflies and moths. Thus tubular flowers adapted to pollination by lepidopterans contain higher levels than flowers fed on by, for instance, flies.[3] Experimental work on butterflies has shown that *Pieris rapae* females prefer nectars containing amino acids over sugar-only nectars.[2]

Some plant species have flowers with two kinds of stamen: some with reproductive anthers that produce normal pollen, and some with 'reward anthers' (Fig. 12.15). Reward anthers are often more conspicuous and brightly coloured to attract potential pollinators, and produce limited quantities of highly nutritious but sterile pollen. They clearly serve to mimic normal anthers and to attract pollinators by deceit.[88] When manoeuvring to forage on them, the insect automatically takes care of pollination with the fertile pollen. Presumably the development of reward anthers is advantageous to the plant in terms of production costs, but this has yet to be proved.

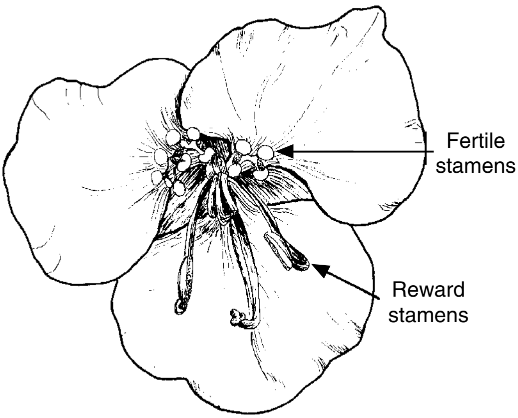


Figure 12.15 Flower of *Commelina tuberosa* with two types of stamen. (From Hess, 1990, with permission.)[49]

12.3.6 Signalling nectar status

Foraging efficiency would be raised if bees, rather than moving randomly between flowers, avoided unrewarding flowers on one hand and recognized rich food sources on the other. Such behaviour has been seen in higher hymenopterans, which assess while still airborne the reward state of a blossom by the smell of 'footprints', volatile pheromones left by previous visitors. Honeybees and bumblebees collecting nectar label their empties by leaving odour traces. The scent marks are of short duration, in the order of minutes, and avoidance of visited flowers by conspecifics as well as heterospecifics is easily observed.[110,118]

Foraging efficiency in bumblebees is still more refined, as exemplified by their capability to adjust, depending on flower species, the duration of avoiding a visited flower. Rejection periods appear to be inversely correlated with nectar secretion rates, which differ greatly among different plant species. The bumblebee *Bombus terrestris*, for example, showed a repellency response of 3–10 min to flowers of *Symphytum officinale* with high nectar secretion rates, whereas repellency of *Melilotus officinalis* and *Lotus corniculatus*, both characterized by relatively low rates of nectar production, lasted minimally 2 and 24 h respectively.[119]

Bees and bumblebees can also deposit a message indicating that the food source is worthwhile to visit. This pheromone is also secreted by the tarsi and was found in bumblebees to consist of a complex mixture of alkanes and alkenes.[111] By recognizing the combination of pheromones left behind by themselves and other bees, they can more easily select the least harvested, most productive flowers. Likewise, stingless bee foragers (Meliponini) deposit attractive odour marks on good food sources to which they recruit[52] and repellent marks on poor food sources that have been exhausted.[89] Clearly, by using such signals, bees improve the efficiency of food-gathering by reducing both the time spent with non-rewarding flowers and the search for rewarding flowers.

On the plant side, something can be gained too by advertising the developmental state of its flowers. Pollination efficiency would increase if the plant signalled to its pollinators which flowers had already been pollinated by previous visitors. Many plants give precisely such a warning by changing flower colour, scent production, and even geometric outline.[117] Thus, the orange flowers of *Lotus scoparius* (Papilionaceae) turn yellow after pollination, whereas the blossoms of some other species change in ultraviolet reflection following pollination.[55] Likewise, pollination results in a local colour change in *Lupinus spinosus* (Papilionaceae). The keel petals of this species possess on their opening a white banner spot which upon pollination turns pink and eventually purple. This colour change seems to be triggered by the growth of pollen tubes into the style.[90]

A spectacular colour change from white to purple takes place in *Viola cornuta* flowers in response to pollination. This change is caused by the synthesis of anthocyanins as a result of an increased expression of three anthocyanin biosynthetic genes. Presumably hormones associated with pollination, such as ethylene and gibberellic acid, lead to the transcriptional activation of these genes and subsequent production of flower pigments.[27]

Flowers also change with age. Floral colours of members from at least 456 species belonging to 78 diverse angiosperm families undergo dramatic, often localized, changes in senescing blossoms (Plate 1).[135] Sexual viability and nectar secretion of postchange flowers is low, because they lack pollen and appear non-receptive. Why, then, do plants keep flowers that have lost their reproductive capacity? It seems likely that retention of older flowers increases a plant's attractiveness to pollinators from a distance. At close range, however, the bees easily learn to discriminate floral colour phases and avoid postchange flowers.[133,134] Thus, by changing their colour in response to pollination or concomitant with ageing, flowers continue to serve the plant by attracting pollinators even after their time is over.

Alterations in the production of fragrances following pollination have been little studied,[117] but some cases involving different families have been reported in the literature.[107,124] For instance, flowers of the orchid *Catasetum maculatum* cease odour production entirely within minutes after pollination, whereas this takes hours (e.g. *Nicotiana attenuata*) to days (e.g. *Platanthera bifolia*) in other species. In this way the plant presumably conserves resources and directs subsequent visitors toward receptive and/or rewarding flowers.[98]

12.4 Pollinator movement within multiple-flower inflorescences

To facilitate flower recognition and thus increase the profit from insect visits, many plant species have their individual blooms clustered into an inflorescence, thereby making a far more conspicuous display than single flowers might achieve. Vertically elongated inflorescences, such as those of foxglove (Fig. 12.16), monkshood, willow herb, and lupin, are of special interest, as this spatial arrangement adds an extra dimension to the pollination economy of monoecious (bisexual) plants.

Within vertical inflorescences bees and flies follow a foraging route that typically starts near the bottom and runs upward. The lower flowers provide more nectar than the upper ones. In agreement with optimal foraging theory, the insects start where the largest nectar source is to be found (i.e. at the base) and, as the distance to an upper flower is small, lower nectar quantities are now acceptable. Moreover, sugar concentration here is often

Figure 12.16 The vertical inflorescence of foxglove (*Digitalis lutea*).

somewhat higher than that in the lower flowers. The upward direction of pollinator movements suits the plant very well, because the flowers of these species (like those of many other plants) are protandrous, that is, the anthers mature some days before the stigmas. Each day a new flower opens at the top of the inflorescence, replacing a senescent flower at the bottom. The older (lower) flowers are functionally female, with receptive stigmas, whereas the top flowers are functionally male, with mature anthers, but still immature stigmas. The foraging behaviour of starting at the bottom and visiting the pollen-containing upper flowers only before leaving the inflorescence obviously promotes cross-pollination and minimizes the chance of self-pollination. The plant's blooming strategy thus seems nicely adapted to pollinator behaviour.[47,77]

12.5 Competition

Insect–flower relationships involve some basic conditions of existence that affect both partners: food availability (to pollinators).and reproductive success (on the plant side). Thus, competition for available resources is likely to occur on both sides. As plant reproductive success is frequently limited by pollinator activity,[10] species will compete for effective pollen carriers, whereas insects will be under selective pressure to exploit their food sources more efficiently than competing species.

Plants can compete for pollinators by producing more flowers. At the same time this increases the risks of geitonogamy (pollination between flowers on the same plant) and sets a limit to pollen export. There are several solutions to this dilemma.[62,115] Plant species can escape competition by utilizing different pollinator species or guilds, for example by differences in floral morphology or by flowering at different times. Adaptation to different pollinator species, as exemplified by high rewards early in the morning so that bumblebees are attracted, or developing long corolla tubes so that only long-tongued insects can reach the nectaries,[99] is undoubtedly a widespread and effective solution. As flowering time is under genetic control, it has been suggested that plant species with a large pollinator overlap avoid competition by blooming at different times. Obviously this resource partitioning and character displacement is mutually beneficial to plants and pollinators. The timing strategy has been observed in some relatively simple plant communities. Thus different plant species in meadow communities in the Rocky Mountains show a regular (i.e. non-random) temporal segregation of blooming periods, thereby reducing competition for bumblebee pollinators.[95] Likewise, most insect-pollinated bog plants use the same species of bumblebee and bloom at different times. A more detailed analysis has revealed that species that depend wholly or to a large extent on bumblebee pollination show a sharper separation in blooming periods than species that are less dependent on bumblebees.[47]

In early spring few plants flower and relatively high numbers of pollinators compete for food. Advancing blooming time may therefore, in addition to the advantage of a longer seed-growth period, be advantageous to a plant through higher pollination success. The risks of freezing, however, may act in the opposite direction.

In late spring and early summer there is a proliferation of blooms. This is nicely reflected in the seasonal changes in the threshold concentration of sucrose solutions that elicit recruitment dances by foraging honeybees. In early summer, when nectar flow is abundant, only high sucrose levels elicit recruitment. In midsummer natural food sources

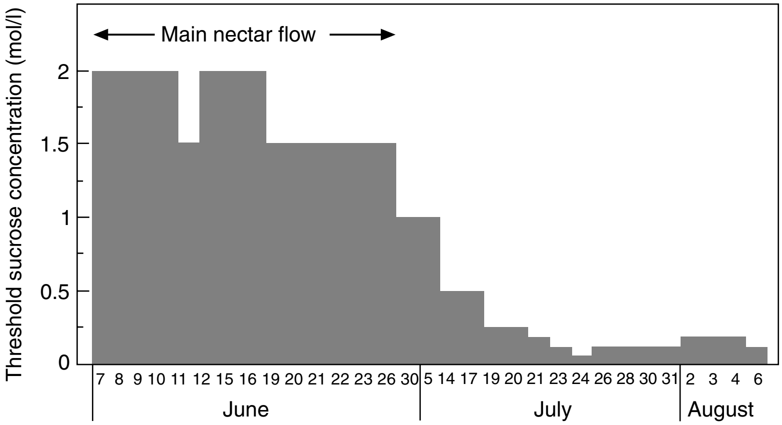


Figure 12.17 Threshold sugar concentrations required to elicit recruitment behaviour in honeybee foragers during early and mid summer. High thresholds coincide with the early summer main nectar flow in flowers (Redrawn from Lindauer, 1948.)[76]

are no longer rich and at the same time have to be shared with many other insects. Now food competition among pollinators is intense. As a result, recruitment dances are elicited by sugar concentrations even 16 times lower than those in late spring (Fig. 12.17). Apparently, bees adjust their acceptance level by force of circumstances and compare food source quality to generally available food.

In springtime some plant species, such as willows (*Salix* spp.) and dandelion (*Taraxacum officinalis*), produce almost endless supplies of nectar and pollen. As many insects feed on such copious food sources, it has been suggested that these plants use a 'dumping' strategy to attract many insects. As a result, seed-setting of competing plant species is reduced and the position of the food supplier is strengthened. Thus, dandelions in an apple orchard can attract pollinators away from apple trees. As a note of irony it may be mentioned that dandelions are largely apomictic, that is, seed development occurs without fertilization. The nonetheless bountiful nectar production must therefore have another function than promotion of reproduction. However, hard evidence for this cunning type of paradox is lacking.

An interesting example of competitive interactions between pollinators relates to two sympatric bumblebee species foraging on two different flower species. Each bee species had an apparent preference for one flower species. However, when all or most of the individuals of either bee species were removed from a local patch, individuals of the remaining bee species would, in addition to their already adopted flower species, start to visit the vacant flower species more frequently.[51]

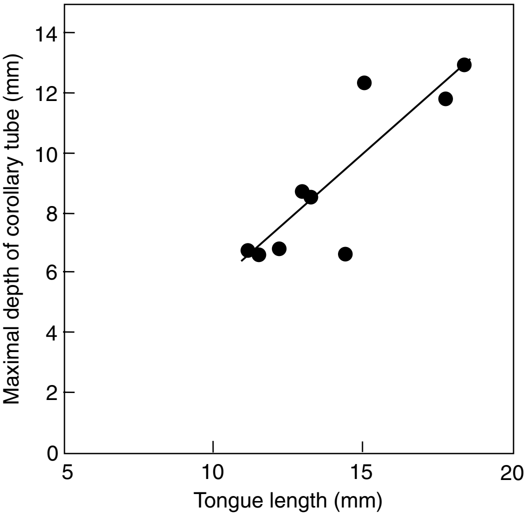


Figure 12.18 Proboscis (= tongue) length versus maximal corolla tube depths visited by queens of nine different bumblebee species. (Data from Ranta and Lundberg, 1980.)[99]

In conclusion, there is strong evidence that competition for pollinators occurs between plants and that the evolutionary outcome of such interactions is resource partitioning (Fig. 12.18)[99] and character displacement. Plants may minimize competition for pollinators by adapting their phenology to the periods during which the chances of fertilization are optimal, as well as evolving structural and physiological characteristics that reduce the spectrum of pollinators, but ensure adequate resources to those they use.[59,87]

12.6 Evolution

Angiosperms are by far the largest present-day group of land plants. They are characterized by a bewildering diversity in flower size, shape, and colour. This conspicuous variation induced Linnaeus to construct a classification of flowering plants in his *Systema Naturae* (1735) based on their sexual organs.

The extraordinary evolutionary success of angiosperms undoubtedly results from adaptations of their reproductive organs to pollination by insects. This pollination system, as deduced from paleobotany and systematics, is an ancient mechanism. The first angiosperms and their sister clades were probably already entomophilous, although some of the early angiosperms may have used both insects and the wind as pollen vectors.[15,18]

The astonishingly rapid radiation of angiosperms towards the end of the Early Cretaceous (between 130 and 90 million years ago), and their takeover of the ancient Mesozoic plant communities of ferns, horsetails, and gymnosperms during the Late Cretaceous and Early Tertiary, has often been linked to a simultaneous diversification of pollen- and nectar-collecting insects during these eras (Fig. 12.19). Many of the sophisticated pollination

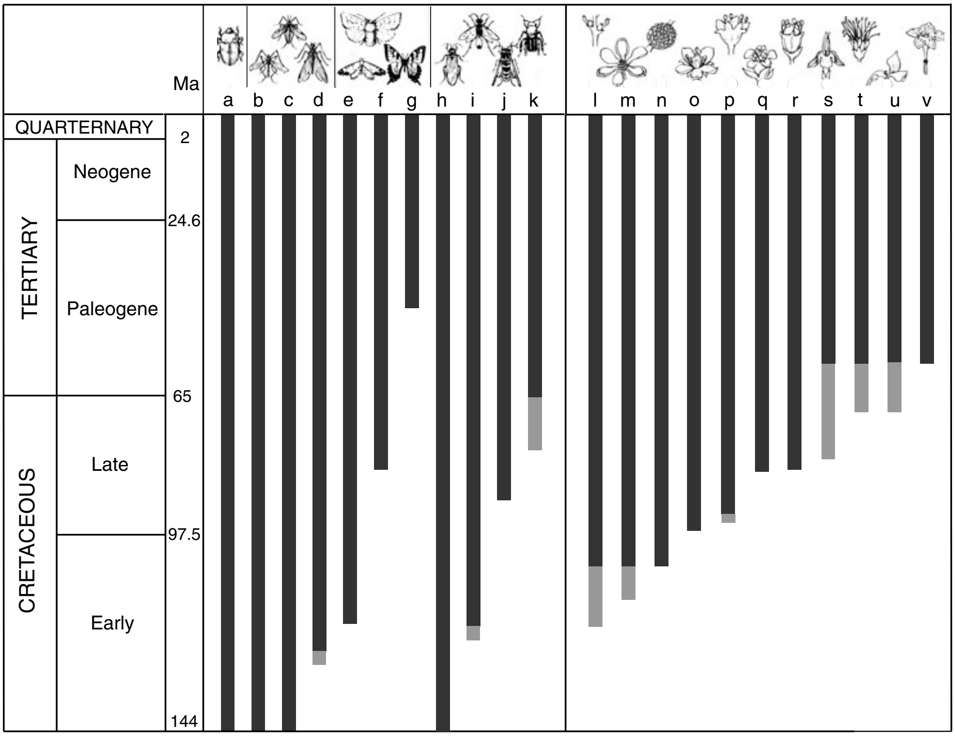


Figure 12.19 Time of appearance on a geological time-scale of selected insect taxa germane to the evolution of insect pollination (a–k) compared with the appearance of major floral types (l–v). Black bars are based on direct fossil evidence; grey bars indicate probable range, inferred rather than based on direct fossil evidence. Ma, million years before present. Insects: (a) Coleoptera; (b–d) Diptera: (b) Tipulidae, (c) Mycethophilidae, (d) Empididae; (e–g) Lepidoptera: (e) Micropterigidae, (f) Noctuidae, (g) Papilionidae; (h–k) Hymenoptera: (h) Symphyta, (i) Sphecidae, (j) Vespidae, (k) Apidae. Plants: (l) small simple flowers with few floral parts, (m) acyclic or hemicyclic flowers with numerous parts; (n) small monochlamydeous flowers; (o) cyclic, heterochlamydeous, and actinomorphic flowers; (p) epigynous and heterochlamydeous flowers; (q) sympetalous flowers; (r) epigynous and monochlamydeous flowers; (s) zygomorphic flowers; (t) brush-type flowers; (u) papilionid flowers; (v) deep funnel-shaped flowers. (Redrawn from Crepet and Friis, 1987;[17] Friis and Crepet, 1987;[31] and Grimaldi, 1996.[44])

systems that characterize extant angiosperms originated at that time.[16]

The advantages of insect pollination compared with wind pollination are manifold. However, disadvantages exist as well, and *anemophily* (wind pollination) has evolved repeatedly from insect-pollinated ancestors;[15,19] many families of insect-pollinated plants contain a few members that have become anemophilous, for example species of *Fraxinus* (Oleaceae), *Thalictrum* (Ranunculaceae), and *Ambrosia* (Asteraceae). In contrast to anemophily, pollination by insect vectors does not require massive and wasteful pollen production and can operate with smaller pollen grains than the most effective size for wind dispersal. Insect pollination also permits effective outcrossing at lower plant population densities and accurate pollen transfer between widely spaced individuals in multispecies vegetations. In some plant communities, such as those found in moist tropical forests, anemophily is almost completely absent because of lack of sufficient air movement (Table 12.4).

Conversely, wind-pollinated species are prevalent in wind-swept temperate regions, such as those of northern latitudes, and in communities of low species diversity. Thus the proportion of anemophilous plants steadily increases with latitude and elevation, reaching 80–100% among the trees of the northernmost regions (Fig. 12.20).[102] Whether reproduction by wind versus insect pollination involves higher energy costs overall still needs to be determined.

As present hymenopterans play a central role in flower pollination, but in the past other groups have been predominant. In the beginning a range of unspecialized insects, with beetles prominent among them, served as pollen carriers. Coleoptera and Diptera are still the primary pollinators of extant basal angiosperms. Lineages of these insects were established by the Late Jurassic and the present-day association of, for example, beetle pollination with primitive woody angiosperms such as *Magnolia* and *Calycanthus* probably goes back to their evolutionary origins.[122] Beetles in some whole genera or even families still feed

Table 12.4 Frequencies (percentage of plant species) of different pollination systems in tropical rainforest trees in Costa Rica and Amazonia, Colombia

Pollen vector	**Costa Rica Lowland Can + Sub (Ref. 58)**	**Costa Rica Lowland Can (Ref. 6)**	**Costa Rica Lowland Sub (Ref. 66)**	**Colombia Flooded Can (Ref. 125)**	**Colombia Upland Can (Ref. 125)**	**Average**
Medium or large bee	29	44	22	24	17	27
Small bee	19	8	17	17	35	19
Beetle	9	—	16	5	4	7
Butterfly	6	2	5	15	12	8
Moth	12	14	7	5	4	8
Wasp	2	4	2	7	3	4
Small insect	19	23	8	14	17	16
Bat	4	4	4	5	2	4
Hummingbird	6	2	18	7	5	8
Wind	2	—	3	3	0	2
Total	110% ($n = 145$)	100% ($n = 52$)	100% ($n = 220$)	100% ($n = 74$)	100% ($n = 68$)	

Two studies present data for different forest strata (Can, canopy; Sub, subcanopy and understorey). Some plant species have more than one pollen vector, causing the cumulative percentage of species to be more than 100%. Despite the fact that the data presented are from different studies, different regions, and different forest types, a general trend can be observed when averaging the results from the five studies.

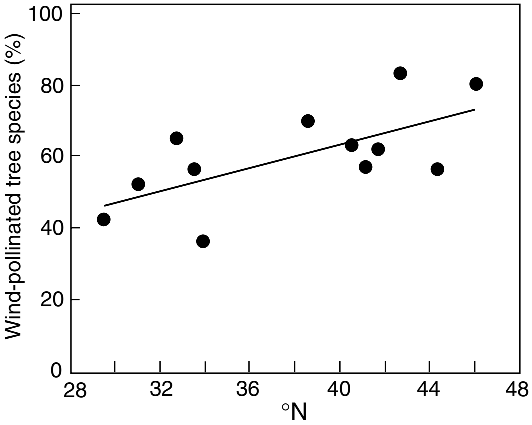


Figure 12.20 Percentage of wind-pollinated tree species in eastern North America as a function of latitude. (From Regal, 1982.)[102]

exclusively on flower parts and, as a side-effect, act as pollinators.

The evolution of angiosperm flower diversity is commonly interpreted as the result of co-evolutionary relationships with pollinating insects.[26] Some insect groups have been more influential than others. Most authors, however, use the term co-evolution to mean reciprocal evolutionary change in interacting species.[123] As regards this definition, the evolutionary relationship between plants and pollinating insects is in the great majority of cases very asymmetrical: the pollinators have decisively influenced the evolution of flowering plants, including extensive radiation in many plant taxa, whereas the plants have hardly affected the evolution (e.g. speciation) of pollinating insects. The asymmetry of this evolutionary relationship becomes less askew when recognizing the fact that pollinators can be clustered into 'functional groups' (e.g. long-tongued flies or small nectar-collecting bees) that exert similar selection pressures, thereby stimulating the development of particular floral traits (e.g. long and narrow corolla tubes or mode of pollen presentation). Convergent selective pressure exerted by functional groups of pollinators may have been an important factor underlying floral diversifiction.[29] Co-evolution in the symmetrical sense has resulted in some exceptionally tight associations, such as between fig and fig wasps and between yuccas and yucca moths.[123]

Other insect groups differ greatly in the intensity of selection pressure they have exerted on flowering plants. A pivotal role in floral evolution accrues to the Apoidea. Because bees are completely dependent on floral resources during both adult and larval stages, they have numerous adaptations to a floral diet. Their digestive system can extract nutrients from pollen grains despite the presence of an almost impermeable cuticle.[127] Few other insect groups have succeeded in exploiting this protein-rich plant product. The well developed learning capacities of Apoidea, together with their advanced flight and navigational abilities, allow for floral constancy and exploitation of widely scattered floral resources.[87]

These features have promoted flower specialization, while flowers, in turn, have evolved structures, such as the floral tube and other corolla characters, that are associated with pollination by bees. Fossil flower remains show that primitive angiosperms had large numbers of stamens, pistils, and petals arranged in a spiral, as in present-day magnolia and white water lily (*Nymphaea alba*) flowers. In the course of time this developed into a regular radial symmetry, and trends towards flower shapes adapted to relationships with particular groups of insects (Fig. 12.21).

These include a reduction in the number of sepals and petals, and the formation of a tubular or spurred corolla with nectaries positioned so that they are accessible only to long-tongued insects. By the Late Cretaceous zygomorphic flower types with one plane of symmetry had evolved. Fusion of flower parts, for instance in papilionid flowers, occurred in the Early Tertiary and a proliferation of advanced floral types reflects the beginning of the spectacular evolutionary interaction between hymenopterans and angiosperms. A bee finds two parts of its body difficult to groom: the areas in the middle of the back and beneath the head. Some zygomorphic flowers, such as certain Fabaceae, exploit this limitation of the bee's dexterity and place their pollen loads on these inaccessible sites, thereby preventing transfer to the pollen baskets.

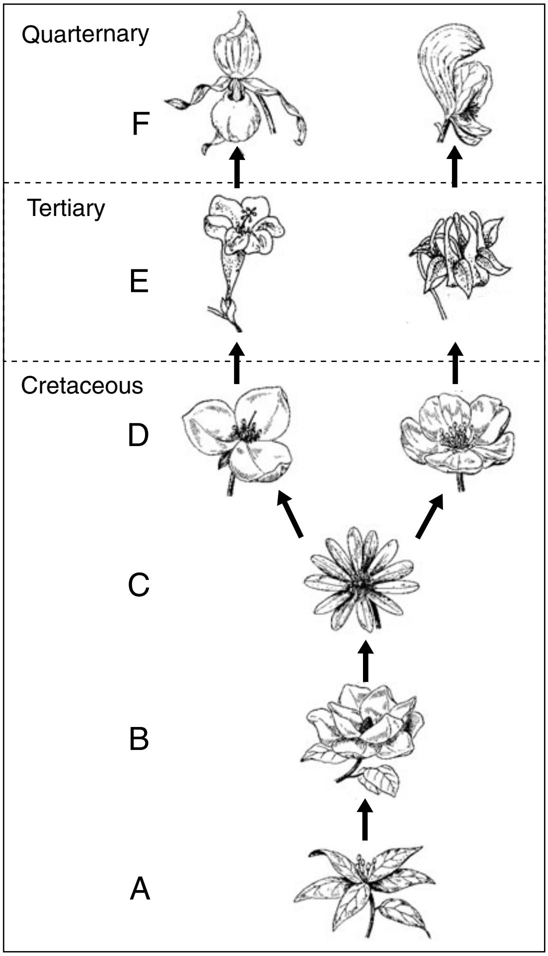


Figure 12.21 Evolutionary trends of flower shapes over 100 million years, as exemplified by extant flowers. (**A**) The earliest flowers had no discernible shape or symmetry. (**B**) Flower of open hemispherical shape, but still without clear symmetry (e.g. *Magnolia* sp). (**C**) Typical open, radially symmetrical flower, such as that of the yellow adonis (*Adonis* sp.). Subsequent divergence often altered shape in monocots (*left*) and dicots (*right*). (**D**) Flowers with reduced but fixed number of floral parts (e.g. spiderwort (*Tradescantia* sp.), *left*, and buttercup (*Ranunculus* sp.), *right*). (**E**) Flowers of increasingly bilateral symmetry and hidden nectaries, as in the freesia (*Freesia* sp.) (*left*) and columbine (*Aquilegia* sp.) (*right*). (**F**) Examples of complex and strongly zygomorphic flower shapes (e.g. the lady's slipper orchid (*Cypripedium* sp.), *left*, and monkshood (*Aconitum* sp.), *right*) (From Leppik, 1971.)[73]

The Late Cretaceous–Early Tertiary was a time of the greatest rate of appearance of new angiosperm taxa, as well as the apparent period of the appearance of bee pollination, suggesting some degree of causal relationship.[18,44,82] The eventual transition to increasingly three-dimensional flower types, like those of orchids and monkshood, probably has two significant advantages. First, because of their conspicuous shapes, pollinators may easily recognize these flowers from a distance, and second, as pollinators will learn how to manoeuvre most efficiently to reach the reward, the position of stamens and pistil can be adapted to the body orientation of the plant's specialized pollinators. In this context it is interesting to note that bumblebees possess innate preferences for bilateral symmetry, so that in their first encounters with flower displays flower-naive bees will prefer to visit and become experienced on bilaterally shaped flowers.[104] A co-evolutionary basis for the origin of bilateral flower lineages and innate preferences for such flower types in bumblebees seems evident.

The advanced position of hymenopterans in the evolutionary association between insects and flowers is supported by the observation that bee-pollinated plant taxa show a greater diversity than taxa dependent on other groups. Thus, bee plants in the southern California flora have an average of 5.9 species per genus, whereas only 3.4 species per genus occur in promiscuous insect-pollinated plants. This difference suggests increased speciation rates in bee plants.[42] Another advantage of pollination by bees is found in their hairy fur. This allows transport of large numbers of pollen grains per visit and the number of ovules in bee-pollinated plant species is accordingly high, resulting in high seed numbers per flower. More than any other group of insects, bees are the driving force of variation in floral design.

The refinement of adaptation to insect pollination culminates in a high multiformity within the Orchidaceae. The monocotyledonous orchids represent the evolutionarily most recent yet the most speciose family of vascular plants, comprising more than 25 000 species. In orchid flowers the pollen grains cohere to form club-shaped pollen packets, called *pollinia*, ususally two to each flower. Each pollinium includes an adhesive tag or clamp, and sticks to the head or another part of the visiting insect. It is then transported to another flower on which, depending on the particular shape of the flower species, the insect lands in such a position that the pollinia are accurately placed on the stigma. One pollinium suffices to fertilize all the ovules from a single pollination, giving rise to many small seeds. The diversity in orchid flower structures represents adaptations to different types of pollinator, about 60% of the orchids being pollinated by bees. Non-social bees in particular, such as bumblebees in the northern hemisphere and solitary euglossine bees in the neotropics, are effective in pollinating widely separated plant populations but ensure outcrossing by the extreme precision of pollen transfer and reception. Even when the pollinating bee visits different orchid species, reproductive isolation is usually maintained because each flower species snaps its pollinia on a different part of the insect's body. Up to 13 different places have been recorded in which the pollinia can be placed.

Orchid diversity is apparent not only in flower shape but also in floral scent. Flower recognition by pollinating insects is promoted not only through great diversity in flower shapes but also by wide variation in floral scents.[57] A bizarre case of floral deception is found in orchids that lure visitors by faking the insect's female sex pheromone. The flowers of about one-third of all orchid species offer neither nectar nor pollen as reward, but produce a scent that mimics the sex attractant of their pollinators. *Ophrys* flowers, for instance, release volatiles that show striking chemical similarities with pheromonal compounds produced by females of their pollinating insects (Fig. 12.22).[93] One step further in this refined deceit mechanism is shown

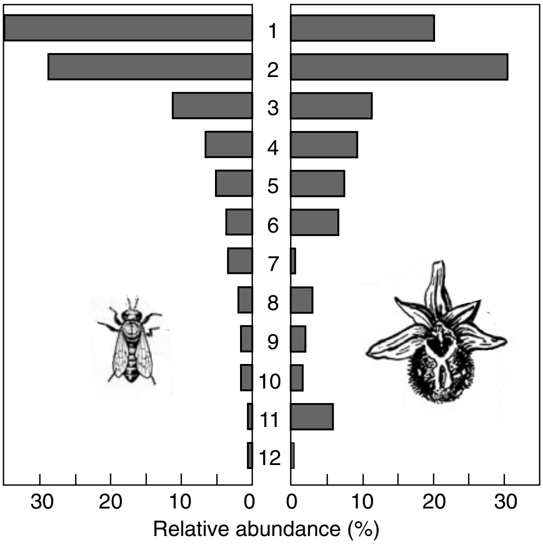


Figure 12.22 A case of a flower bouquet mimicking an insect's pheromone. The composition of the sex pheromone of a pollinating bee (*Andrena nigroaenea*) is compared to that of the floral scent of the orchid *Ophrys sphegodes*. Cuticle extracts of virgin female bees (*left*) contain 12 straight-chain saturated and unsaturated hydrocarbons (numbered 1–12), which occur also in labellum extracts of *O. sphegodes* (*right*) in roughly similar proportions. (Data from Schiestl *et al.*, 1999.)[109]

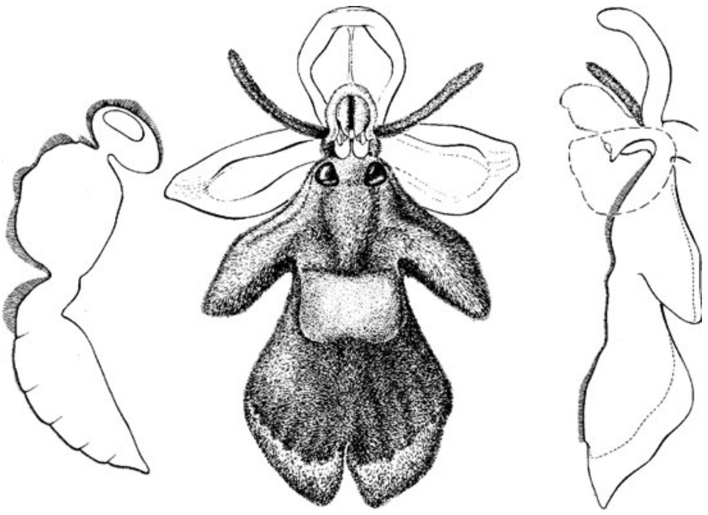


Figure 12.23 The hairy coverings of the fly orchid *Ophrys insectifera* and its pollinator, the wasp *Gorytes mystaceus* (*left*). On the right is the flower contour, with hairs, showing their arrangement and the direction of the nap. The resemblance to a female wasp is increased by the coloration of the flower's labellum. (From Kullenberg, 1961.)[68]

by *Ophrys sphegodes* flowers, which upon pollination increase the production of a compound mimicking the signal substance produced by females of their pollinators after copulation. This compound, farnesyl hexanoate, inhibits copulation with fertilized females by male bees and, likewise, reduces visitation of pollinated flowers.[108]

Many *Ophrys* flowers, in addition to the odorous lure, have developed visual and tactile stimuli to mimic conspecific female insects (Fig. 12.23). Patrolling males become sexually excited and upon landing attempt to mate with the flower. Such 'pseudo-copulations' rarely lead to the release of sperm but bring the male to touch the pollinia, which become attached to its body. Pollination may occur when the insect is attracted to another flower. This strategy has the advantage that visiting a flower does not extinguish the insect's sex drive and the next flower remains as attractive as the previous one. These cases may be regarded as a kind of 'behavioural parasitism' on the part of the plant, because the insect is exploited without a reward. This tactic is not a unique exception. It has evolved independently at least three times among the orchids and their visitors, and occurs occasionally in other plant taxa as well, involving various insect groups.[120]

12.7 Nature conservation

As insect pollination is central to maintenance of the plant diversity of world ecosystems any significant reduction in natural pollinators may have devastating effects on the plant world. Bees in particular play a paramount role. As aptly stated by Neff and Simpson:[87]

> there can be little doubt that bees are extremely important, or the most important, group of pollinators in a wide array of plant communities. Indeed it is difficult to envision a world without bees. Other insects [. . .] might be able to assume the role of bees in some cases but in many communities, large proportions of the flora [. . .] are obligately dependent on bees as pollinators. Many of these plants [. . .] would simply disappear if bees were suddenly eliminated from the systems in which they occur.

Despite this notion, conservation ecologists have very few quantitative data on the effects of changes in the pollinator force on plant communities. Two unintentional large-scale 'experiments' may be cited to show that when native pollinator populations decline seed-set in some plant species in natural habitats or agrosystems is reduced. In the early 1970s large forested areas of New Brunswick in Canada were sprayed with an insecticide that was highly toxic to bees, to control an outbreak of spruce budworm (*Choristoneura fumiferana*). This severely affected pollination success in blueberry fields. When the use of this insecticide was discontinued, a steady recovery could be seen.[59,61] Misuse of diazinon for aphid control on alfalfa fields in northwestern parts of the USA killed most alkali bees in 1973. More than 2 years later alkali bees had regained only 25% of their initial populations.[54] Likewise, large-scale uses of herbicides, which remove alternative food sources for pollinators, may have far-reaching implications for natural vegetations via negative effects on wild insect pollinators.

Habitat destruction, including the removal of marginal lands and hedgerows, leads to a reduced diversity of forage plants and nest sites of natural pollinators, and is therefore a major cause of the alarming decline in the diversity and numbers of native bees.[11] This decline, in turn, may feed back on the local flora.

Another form of habitat modification caused by human activities is habitat fragmentation. The viability of plant populations may be affected by local habitat fragmentation through reduced insect pollination. Such effects have been observed in field felwort (*Gentianella campestris*) plants occurring in large and small local habitat fragments in grassland sites. In this case extinction rates were found to increase with increased local fragmentation, due to reduced cross-pollination levels. In large local habitat fragments, flower visitation rates by bumblebees appeared to be four to six times greater than those in small fragments. As a result, seed set and seed quality in small fragments was markedly reduced, causing significant differences in plant population viability.[72]

The introduction of honeybees or bumblebees to a foreign place undoubtedly bears some risks to native pollinators. In Tasmania, for instance, by far the most abundant flower-visiting insect at almost every site is the introduced honeybee, often outnumbering all other flower-visiting insects by a factor of 10 or more. The majority of floral resources are gathered by these bees, often during the morning before native bees have become active. Very likely only a fraction of the available floral resources has remained accessible to the local pollinators. This must have had a great impact on the population numbers and even survival of local pollinator species, which in turn has possibly also affected the local flora. Thus, the introduction of bees in an area beyond their home range may have important consequences for local biodiversity.[39]

It is now recognized that pollinators—wild species as well as honeybees—suffer worldwide from habitat destruction, insecticide poisonings, and the spread of parasites and pathogens. Therefore a global pollination crises seems imminent, with severe impact not only on natural ecosystems, but also on agricultural production.[61]

12.8 Economy

An inconspicuous but pivotal contribution of insects to human food sources is their pollination of crop plants. About 30% of our food is derived from bee-pollinated plants. The pollination efficiency of bees is amazingly high. One hundred honeybees, for instance, can set a commercial crop of one hectare of apples in 5 h. The role of honeybees as honey producers is minute in comparison. On a world basis the value of crops pollinated by bees exceeds the value of the annual honey crop by a factor of 50. Exact figures for crop losses following the removal of all honeybees are hard to determine, but it is estimated that the value of crop pollination by honeybees amounts to US$5–14 billion annually for 63 crops in the USA alone.[65] The economic value of honeybee pollination of 177 crops in the European Union amounts to roughly €4 billion.[137] Poor pollination levels not only reduce crop yields but, equally importantly, they reduce the quality of crops such as apples, melons, and other fruits.

Often, approximately 80% of the insect pollination of crops in the Western world is attributed to honeybees, but this figure may be an overestimation and the contribution of honeybees may be considerably lower. Repeated studies have shown that for many crops native bees are either an important adjunct to honeybees as pollinators or are even superior to them.[65,92] The decline of wild bee species, which has been well documented for several parts of Europe and North America, is therefore a matter of serious concern with regard to future agricultural production.[61] Maintaining some uncultivated land areas as refuge habitats (25% of the total[4]) could stop a further decline of unmanaged bees and at the same time provide havens for insect natural enemies, which are beneficial in the control of pest species. Some recent examples of such measures with positive results are encouraging.[65, 92]

Even self-pollinating crop plant species may produce considerably higher yields when grown near a good pollinator habitat. Coffee shrubs, for instance, show up to 50% higher yields in regions with stable native or introduced bee populations.[105] As coffee is grown in many of the world's most biodiverse and threatened regions, the finding that forest-based pollinators increase coffee yield considerably illustrates the potential economic value of forest conservation in agricultural landscapes.[103]

To compensate for local shortages in natural pollinators, large numbers of honeybee colonies are often rented and moved, sometimes over great distances.[30,53] Additional pollination capacity can be obtained by rearing other bee species. These are nowadays produced and distributed on a commercial scale to enhance pollination success, either in the open field (leafcutter bees) or in greenhouses (bumblebees).

12.9 Conclusions

The fascinating panorama of partnerships that exist between insects and flowers provides a window on one of the longest relationships in biological history. It shows at the same time a range and complexity unsurpassed by any other type of interaction between insects and plants. This complexity arises from the interplay of two dynamic systems. Superimposed on mutualism between the plants and their pollinators, the same two partners form competitively interacting systems of (1) plants for pollinators and (2) pollinators for floral resources.[59] The outcome of this complex interplay is often hard to predict but is at the heart of the present-day composition of the Earth's biota. The terrestrial ecosystems, as we know them, would probably never have reached their present richness in the absence of pollinating insects.

The relationships between flowers and pollinators have been the subject of many books. As a well written and delightfully illustrated example, F. G. Barth's book *Insects and Flowers*,[5] may be mentioned. Equally informative and superbly written introductions are those by Gould and Gould,[37] Heinrich,[47] Proctor *et al.*,[96] and the recent elegant book by Goulson.[40] Dafni has provided a useful manual of the methods and procedures used in pollination research, with an emphasis on ecological studies.[21] Thompson has presented a thorough review of the principles of co-evolution and mutualism.[123]

12.10 References

1. Adler, L.S. (2000). The ecological significance of toxic nectar. *Oikos*, **91**, 409–20.
2. Alm, J., Ohnmeiss, T.E., Lanza, J., and Vriesenga, L. (1990). Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. *Oecologia*, **84**, 53–7.
3. Baker, H.G. and Baker, I. (1986). The occurrence and significance of amino acids in floral nectars. *Plant Systematics and Evolution*, **151**, 175–86.
4. Banaszak, J. (2000). Effect of habitat heterogeneity on the diversity and density of pollinating insects. In *Interchanges of insects between agricultural and surrounding landscapes* (ed. B. Ekbom, M.E. Irwin, and Y. Robert), pp. 123–40. Kluwer, Dordrecht.
5. Barth, F.G. (1985). *Insects and flowers. The biology of a partnership*. Princeton University Press, Princeton, NJ.
6. Bawa, K.S., Bullock, S.H., Perry, D.R., Coville, R.E., and Grayum, M.H. (1985). Reproductive biology of tropical lowland rain forest trees. II. Pollination systems. *American Journal of Botany*, **72**, 346–56.
7. Beekman, M. and Ratnieks, F.L.W. (2000). Long-range foraging by the honey-bee, *Apis mellifera* L. *Functional Ecology*, **14**, 490–6.
8. Boch, R. (1956). Die Tänze der Biene bei nahen und fernen Trachtquellen. *Zeitschrift für Vergleichende Physiologie*, **38**, 136–67.
9. Braun, E., MacVicar, R.M., Gibson, D.A., Pankiw, P., and Guppy, J. (1953). Studies in red clover seed production. *Canadian Journal of Agricultural Science*, **3**, 48–53.
10. Burd, M. (1994). Bateman's principle and plant recognition: the role of pollen limitation in fruit and seed set. *Botanical Review*, **60**, 83–139.
11. Cane, J. H. (2001). Habitat fragmentation and native bees: a premature verdict? *Conservation Ecology*, **5**, article no. 3.
12. Chittka, L. (1997). Bee color vision is optimal for coding flower color, but flower colors are not optimal for being coded—why? *Israel Journal of Plant Sciences*, **45**, 115–27.
13. Chittka, L. and Waser, N.M. (1997). Why red flowers are not invisible to bees. *Israel Journal of Plant Sciences*, **45**, 169–83.
14. Cook, J.M. and Rasplus, J.Y. (2003). Mutualists with attitude: coevolving fig wasps and figs. *Trends in Ecology and Evolution*, **18**, 241–8.
15. Cox, P.A. (1991). Abiotic pollination: an evolutionary escape for animal-pollinated angiosperms. *Philosophical Transactions of the Royal Society B*, **333**, 217–24.
16. Crane, P.R., Friis, E.M., and Pedersen, K.R. (1995). The origin and early diversification of angiosperms. *Nature*, **374**, 27–33.
17. Crepet, W.L. and Friis, E.M. (1987). The evolution of insect pollination in angiosperms. In *The origins of Angiosperms and their biological consequences* (ed. E.M. Friis, W.G. Chalconer, and P.R. Crane), pp. 181–201. Cambridge University Press, Cambridge.
18. Crepet, W.L., Friis, E.M, and Nixon, K.C. (1991). Fossil evidence for the evolution of biotic pollination. *Philosophical Transactions of the Royal Society B*, **333**, 187–95.
19. Culley, T.M., Weller, S.G., and Sakai, A.K. (2002). The evolution of wind pollination in angiosperms. *Trends in Ecology and Evolution*, **17**, 361–9.
20. Dafni, A. (1984). Mimicry and deception in pollination. *Annual Review of Ecology and Systematics*, **15**, 259–78.
21. Dafni, A. (1992). *Pollination ecology. A practical approach*. IRL Press, Oxford.
22. Darwin, C. (1859). *The origin of species*. John Murray, London.
23. De La Barrera, E. and Nobel, P.S. (2004). Nectar: properties, floral aspects, and speculations on origin. *Trends in Plant Science*, **9**, 65–9.
24. Dobson, H.E.M. (1994). Floral volatiles in insect biology. In *Insect–plant interactions*, Vol. 5 (ed. E.A. Bernays), pp. 47–81. CRC Press, Boca Raton, FL.
25. Dobson, H.E.M., Bergström, G., and Groth, I. (1990). Differences in fragrance chemistry between flower parts of *Rosa rugosa* Thunb. (Rosaceae). *Israel Journal of Botany*, **39**, 143–56.
26. Dodd, M.E., Silvertown, J., and Chase, M.W. (1999). Phylogenetic analysis of trait evolution and species

diversity variation among angiosperm families. *Evolution*, **53**, 732–44.

27. Farzad, M., Griesbach, R., Hammond, J., Weiss, M.R., and Elmendorf, H.G. (2003). Differential expression of three key anthocyanin biosynthetic genes in a color-changing flower, *Viola cornuta* cv. Yesterday, Today and Tomorrow. *Plant Science*, **165**, 1333–42.
28. Feinsinger, P. (1983). Coevolution and pollination. In *Coevolution* (ed. D.J. Futuyma and M. Slatkin), pp. 282–310. Sinauer, Sunderland, MA.
29. Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R., and Thomson, J.D. (2004). Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics*, **35**, 375–403.
30. Free, J.B. (1993). *Insect pollination of crops* (2nd edn). Academic Press, London.
31. Friis, E.M. and Crepet, W.L. (1987). Time of appearance of floral features. In *The origins of angiosperms and their biological consequences* (ed. E.M. Friis, W.G. Chalconer, and P.R. Crane), pp. 145–79. Cambridge University Press, Cambridge.
32. Frisch, K. von (1967). *The dance language and orientation of bees*. Harvard University Press, Cambridge.
33. Gardener, M.C. and Gillman, M.P. (2002). The taste of nectar—a neglected area of pollination ecology. *Oikos*, **98**, 552–7.
34. Gegear, R.J. and Laverty, T.M. (1995). Effect of flower complexity on relearning flower-handling skills in bumble bees. *Canadian Journal of Zoology*, **73**, 2052–8.
35. Gilbert, F.S., Haines, H., and Dickson, K. (1991). Empty flowers. *Functional Ecology*, **5**, 29–39.
36. Giurfa, M., Dafni, A., and Neal, P.R. (1999). Floral symmetry and its role in plant–pollinator systems. *International Journal of Plant Sciences*, **160**, S41–S50.
37. Gould, J.L. and Gould, C.G. (1988). *The honey bee*. W.H. Freeman, New York.
38. Goulson, D. (2000). Are insects flower constant because they use search images to find flowers? *Oikos*, **88**, 547–52.
39. Goulson, D. (2003). Effects of introduced bees on native ecosystems. *Annual Review of Ecology, Evolution and Systematics*, **34**, 1–26.
40. Goulson, D. (2003a). *Bumblebees. Their behaviour and ecology*. Oxford University Press, Oxford.
41. Goulson, D., Stout, J.C., and Hawson, S.A. (1997). Can flower constancy in nectaring butterflies be explained by Darwin's interference hypothesis? *Oecologia*, **112**, 225–31.
42. Grant, V. (1949). Pollination systems as isolating mechanisms in angiosperms. *Evolution*, **3**, 82–97.
43. Grant, V. (1950). The flower constancy of bees. *Botanical Review*, **16**, 379–98.
44. Grimaldi, D.A. (1996). Captured in amber. *Scientific American*, **274**(4), 71–7.
45. Heinrich, B. (1976). The foraging specializations of individual bumblebees. *Ecological Monographs*, **46**, 105–28.
46. Heinrich, B. (1979a). 'Majoring' and 'minoring' by foraging bumblebees, *Bombus vagans*: an experimental analysis. *Ecology*, **60**, 245–55.
47. Heinrich, B. (1979b). *Bumblebee economics*. Harvard University Press, Cambridge, MA.
48. Heinrich, B. and Raven, P.H. (1972). Energetics and pollination ecology. *Science*, **176**, 597–602.
49. Hess, D. (1990). *Die Blüte* (2nd edn). Ulmer, Stuttgart.
50. Higginson, A.D. and Barnard, C.J. (2004). Accumulating wing damage affects foraging decisions in honeybees (*Apis mellifera* L.). *Ecological Entomology*, **29**, 52–9.
51. Inouye, D.W. (1978). Resource partitioning in bumblebees: experimental studies of foraging behavior. *Ecology*, **59**, 672–8.
52. Jarau, S., Hrncir, M., Ayasse, M., Schulz, C., Francke, W., Zucchi, R., *et al*. (2004). A stingless bee (*Melipona seminigra*) marks food sources with a pheromone from its claw retractor tendons. *Journal of Chemical Ecology*, **30**, 793–804.
53. Jay, S.C. (1986). Spatial management of honey bees on crops. *Annual Review of Entomology*, **31**, 49–65.
54. Johansen, C.A. (1977). Pesticides and pollinators. *Annual Review of Entomology*, **22**, 177–92.
55. Jones, C.E. and Buchmann, S.L. (1974). Ultraviolet floral patterns as functional orientation cues in hymenopterous pollination systems. *Animal Behaviour*, **22**, 481–5.
56. Jürgens, A., Witt, T., and Gottsberger, G. (2003). Flower scent composition in *Dianthus* and *Saponaria* species (Caryophyllaceae) and its relevance for pollination biology and taxonomy. *Biochemical Systematics and Ecology*, **31**, 345–57.
57. Kaiser, R. (1993). *The scents of orchids. Olfactory and chemical investigations*. Elsevier, Amsterdam.
58. Kang, H. and Bawa, K.S. (2003). Effects of successional status, habit, sexual systems, and pollinators on flowering patterns in tropical rain forest trees. *American Journal of Botany*, **90**, 865–76.
59. Kevan, P.G. and Baker, H.G. (1983). Insects as flower visitors and pollinators. *Annual Review of Entomology*, **28**, 407–53.
60. Kevan, P.G. and Lane, M. (1985). Flower petal microtexture is a tactile cue for bees. *Proceedings of the National Academy of Sciences of the USA*, **82**, 4750–2.
61. Kevan, P.G. and Phillips T.P. (2001). The economic impacts of pollinator declines: an approach to

assessing the consequences. *Conservation Ecology*, **5**, article no. 8.

62. Klinkhamer, P.G.L. and de Jong, T.J. (1993). Attractiveness to pollinators: a plant's dilemma. *Oikos*, **66**, 180–4.
63. Knoll, F. (1956). *Die Biologie der Blüte*. Springer, Berlin.
64. Knudsen, J.T., Tollsten, L., and Bergström, G. (1993). Floral scents—a checklist of volatile compounds isolated by head-space techniques. *Phytochemistry*, **33**, 253–80.
65. Kremen, C., Williams N.M., and Thorp, R.W. (2002). Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences of the USA*, **99**, 16812–16.
66. Kress, W.J. and Beach, J.H. (1990). Cited in: Bawa, K.S. (1990). Plant–pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics*, **21**, 399–422.
67. Kriston, I. (1971). Zum Problem des Lernverhaltens von *Apis mellifica* L. gegenüber verschiedenen Duftstoffen. *Zeitschrift für Vergleichende Physiologie*, **74**, 169–89.
68. Kullenberg, B. (1961). Studies on *Ophrys* L. pollination. *Zoologiska Bidrag från Uppsala*, **34**, 1–340.
69. Laloi, D., Bailez, O., Blight, M.M., Roger B., Pham-Delègue, H.M., and Wadhams, L.J. (2000). Recognition of complex odors by restrained and free-flying honeybees, *Apis mellifera*. *Journal of Chemical Ecology*, **26**, 2307–19.
70. Laverty, T.M. (1994). Bumble bee learning and flower morphology. *Animal Behaviour*, **47**, 531–45.
71. Laverty, T.M. and Plowright, R.C. (1988). Flower handling by bumblebees: a comparison of specialists and generalists. *Animal Behaviour*, **36**, 733–40.
72. Lennartsson, T. (2002). Extinction thresholds and disrupted plant–pollinator interactions in fragmented plant populations. *Ecology*, **83**, 3060–72.
73. Leppik, E.E. (1971). Origin and evolution of bilateral symmetry in flowers. *Evolutionary Biology*, **5**, 49–86.
74. Lewis, A.C. (1993). Learning and the evolution of resources: pollinators and flower morphology. In *Insect learning. Ecological and evolutionary perspectives* (ed. D.R. Papaj and A.C. Lewis), pp. 219–42. Chapman & Hall, New York.
75. Lewis, A.C. and Lipani, G. (1990). Learning and flower use in butterflies: hypotheses from honey bees. In *Insect–plant interactions*, Vol. 2 (ed. E.A. Bernays), pp. 95–110. CRC Press, Boca Raton, FL.
76. Lindauer, M. (1948). Über die Einwirkung von Duft- und Geschmacksstoffen sowie anderer Faktoren auf die Tänze der Bienen. *Zeitschrift für Vergleichende Physiologie*, **31**, 348–412.
77. McKone, M.J., Ostertag, R., Rausher, J.T., Heiser, D.A., and Russell, F.L. (1995). An exception to Darwin's syndrome: floral position, protogyny, and insect visitation in *Besseya bullii* (Scrophulariaceae). *Oecologia*, **101**, 68–74.
78. Maynard Smith, J. (1982). *Evolution and the theory of games*. Cambridge University Press, Cambridge.
79. Menzel, R. and Shmida, A. (1993). The ecology of flower colours and the natural colour vision of insect pollinators: the Israeli flora as a study case. *Biological Reviews*, **68**, 81–120.
80. Menzel, R., Erber, I., and Masuhr, T. (1974). Learning and memory in the honeybee. In *Experimental analysis of insect behaviour* (ed. L. Barton Browne), pp. 195–217. Springer, Berlin.
81. Mevi-Schütz, J., Goverde, M., and Erhardt, A. (2003). Effects of fertilization and elevated CO_2 on larval food and butterfly nectar amino acid preference in *Coenonympha pamphilus* L. *Behavioral Ecology and Sociobiology*, **54**, 36–43.
82. Michener, C.D. and Grimaldi, D.A. (1988). The oldest fossil bee: apoid history, evolutionary stasis, and antiquity of social behavior. *Proceedings of the National Academy of Sciences of the USA*, **85**, 6424–6.
83. Moore, P.D., Webb, J.A., and Collinson, M.E. (1991). *Pollen analysis* (2nd edn). Blackwell, Oxford.
84. Morse, R.A. (1958). The pollination of bird's-foot trefoil. *Proceedings of the Tenth International Congress of Entomology*, **4**, 951–3.
85. Nason, J.D., Herre, E.A., and Hamrick, J.L. (1998). The breeding structure of a tropical keystone plant resource. *Nature*, **391**, 685–7.
86. Ne'eman, G., Dafni, A., and Potts, S.G. (1999). A new pollination probability index (PPI) for pollen load analysis as a measure for pollination effectiveness of bees. *Journal of Apicultural Research*, **38**, 19–24.
87. Neff, J.L. and Simpson, B.B. (1993). Bees, pollination systems and plant diversity. In *Hymenoptera and biodiversity* (ed. J. LaSalle and I.D. Gauld), pp. 143–67. CAB International, Wallingford, UK.
88. Nepi, M., Guarnieri, M., and Pacini, E. (2003). 'Real' and feed pollen of *Lagerstroemia indica*: ecophysiological differences. *Plant Biology*, **5**, 311–14.
89. Nieh, J.C., Ramírez, S., and Nogueira-Neto, P. (2003). Multi-source odor-marking of food by a stingless bee, *Melipona mandacaia*. *Behavioral Ecology and Sociobiology*, **54**, 578–86.
90. Nuttman, C. and Willmer, P. (2003). How does insect visitation trigger floral colour change? *Ecological Entomology*, **28**, 467–74.
91. Olesen, J.M. and Warncke, E. (1989). Flowering and seasonal changes in flower sex ratio and frequency

of flower visitors in a population of *Saxifraga hirculus*. *Holarctic Ecology*, **12**, 21–30.

92. O'Toole, C. (1993). Diversity of native bees and agrosystems. In *Hymenoptera and biodiversity* (ed. J. LaSalle and I.D. Gauld), pp. 169–96. CAB International, Wallingford, UK.
93. Paxton, R.J. and Tengö, J. (2001). Double duped males: the sweet and the sour of the orchid's bouquet. *Trends in Ecology and Evolution*, **16**, 167–9.
94. Pham-Delègue, M.H., Etievant, P., Guichard, E., Marilleau, R., Douault, P., Chauffaille, J., *et al*. (1990). Chemicals involved in honeybee–sunflower relationship. *Journal of Chemical Ecology*, **16**, 3053–65.
95. Pleasants, J.M. (1980). Competition for bumblebee pollinators in Rocky Mountain plant communities. *Ecology*, **61**, 1446–59.
96. Proctor, M., Yeo, P., and Lack, A. (1996). *The natural history of pollination*. Harper Collins, London.
97. Raguso, R.A. (2004a). Why are some floral nectars scented? *Ecology*, **85**, 1486–94.
98. Raguso, R.A. (2004b). Why do flowers smell? The chemical ecology of fragrance-driven pollination. In *Advances in insect chemical ecology* (ed. R.T. Cardé and J.G. Millar), pp.151–78. Cambridge University Press, Cambridge.
99. Ranta, E. and Lundberg, H. (1980). Resource partioning in bumblebees: the significance of differences in proboscis length. *Oikos*, **35**, 298–302.
100. Reader, R.J. (1975). Competitive relationships of some bog ericads for major insect pollinators. *Canadian Journal of Botany*, **53**, 1300–5.
101. Real, L., Ott, J., and Silverline, E. (1982). On the trade-off between the mean and the variance in foraging: effects of spatial distribution and color preference. *Ecology*, **63**, 1617–23.
102. Regal, P.J. (1982). Pollination by wind and animals: ecology and geographic patterns. *Annual Review of Ecology and Systematics*, **13**, 497–524.
103. Ricketts, T.H., Daily, G.C., Ehrlich, P.R., and Michener, C.D. (2004). Economic value of tropical forest to coffee production. *Proceedings of the National Academy of Sciences of the USA*, **101**, 12579–82.
104. Rodríguez, I., Gumbert, A., Hempel de Ibarra, N., Kunze, J., and Giurfa, M. (2004). Symmetry is in the eye of the 'beeholder': innate preference for bilateral symmetry in flower-naive bumblebees. *Naturwissenschaften*, **91**, 374–7.
105. Roubik, D.W. (2002). The value of bees to the coffee harvest. *Nature*, **417**, 708.
106. Roulston, T.A.H., Cane, J.H., and Buchmann, S.L. (2000). What governs protein content of pollen: pollinator preferences, pollen–pistil interactions, or phylogeny? *Ecological Monographs*, **70**, 617–43.
107. Sazima, M., Vogel, S., Cocucci, A., and Hausner, G. (1993). The perfume flowers of *Cyphomandra* (Solanaceae): pollination by euglossine bees, bellows mechanism, osmophores, and volatiles. *Plant Systematica and Evolution*, **187**, 51–88.
108. Schiestl, F.P. and Ayasse, M. (2001). Post-pollination emission of a repellent compound in a sexually deceptive orchid: a new mechanism for maximising reproductive success? *Oecologia*, **126**, 531–4.
109. Schiestl, F.P., Ayasse, M., Paulus, H.F., Löfstedt, C., Hansson, B.S., Ibarra, F., *et al*. (1999). Orchid pollination by sexual swindle. *Nature*, **399**, 421–2.
110. Schmitt, U. and Bertsch, A. (1990). Do foraging bumblebees scent-mark food sources and does it matter? *Oecologia*, **82**, 137–44.
111. Schmitt, U., Lübke, G., and Francke, W. (1991). Tarsal secretion marks food sources in bumblebees (Hymenoptera: Apidae). *Chemoecology*, **2**, 35–40.
112. Schnetter, B. (1972). Experiments on pattern discrimination in honey bees. In *Information processing in the visual system of arthropods* (ed. R. Wehner), pp. 195–201. Springer, Berlin.
113. Seeley, T.D. (1985). *Honeybee ecology*. Princeton University Press, Princeton, NJ.
114. Snodgrass, R.E. (1956). *Anatomy of the honey bee*. Comstock, Ithaca, NY.
115. Snow, A.A., Spira, T.P., Simpson, R., and Klips, R.A. (1996). The ecology of geitonogamous pollination. In *Floral biology. Studies on floral evolution in animal-pollinated plants* (ed. G. Lloyd and S.C.H. Barrett), pp. 191–216. Chapman & Hall, New York.
116. Sprengel, C.K. (1793). *Das entdeckte Geheimniss der Natur im Bau und in der Befruchtung der Blumen*. F. Vieweg, Berlin (reprinted 1972 by J. Cramer, Lehre, and Weldon & Wesley, Codicote, UK and New York. An English translation of the general part of this book by P. Haase has been published in *Floral biology. Studies on floral evolution in animal-pollinated plants* (ed. D.G. Lloyd and S.C.H. Barrett), 1996, pp. 3–43. Chapman & Hall, New York.)
117. Stead, A.D. (1992). Pollination-induced flower senescence: a review. *Plant Growth Regulation*, **11**, 13–20.
118. Stout, J.C. and Goulson, D. (2001). The use of conspecific and interspecific scent marks by foraging bumblebees and honeybees. *Animal Behaviour*, **62**, 183–9.
119. Stout, J.C. and Goulson, D. (2002). The influence of nectar secretion rates on the responses of bumblebees (*Bombus* spp.) to previously visited flowers. *Behavioral and Ecological Sociobiology*, **52**, 239–46.

120. Stowe, M.K. (1988). Chemical mimicry. In *Chemical mediation of coevolution* (ed. K.C. Spencer), pp. 513–80. Academic Press, San Diego.
121. Thakar, J.D., Kunte, K., Chauhan, A.K., Watve, A.V., and Watve, M.G. (2003). Nectarless flowers: ecological correlates and evolutionary stability. *Oecologia*, **136**, 565–70.
122. Thien, L.B., Azuma, H., and Kawano, S. (2000). New perspectives on the pollination biology of basal angiosperms. *International Journal of Plant Sciences*, **161**, S225–35.
123. Thompson, J.N. (1994). *The coevolutionary process*. University of Chicago Press, Chicago.
124. Tollsten, L. and Bergström, G. (1989). Variation and post-pollination changes in floral odours released by *Platanthera bifolia* (Orchidaceae). *Nordic Journal of Botany*, **9**, 359–62.
125. Van Dulmen, A. (2001). Pollination and phenology of flowers in the canopy of two contrasting rain forest types in Amazonia, Colombia. *Plant Ecology*, **153**, 73–85.
126. Vainstein, A., Lewinsohn, E., Pichersky, E., and Weiss, D. (2001). Floral fragrance. New inroads into an old commodity. *Plant Physiology*, **127**, 1383–9.
127. Velthuis, H.W.W. (1992). Pollen digestion and the evolution of sociality in bees. *Bee World*, **73**, 77–89.
128. Vogel, S. (1996). Christian Konrad Sprengel's theory of the flower: the cradle of floral ecology. In *Floral biology. Studies on floral evolution in animal-pollinated plants* (ed. D.G. Lloyd and S.C.H. Barrett), pp. 44–62. Chapman & Hall, New York.
129. Waddington, K.D. (1985). Cost-intake information used in foraging. *Journal of Insect Physiology*, **31**, 891–7.
130. Waddington, K.D. (1990). Foraging profits and thoracic temperature of honey bees (*Apis mellifera*). *Journal of Comparative Physiology B*, **160**, 325–9.
131. Walther-Hellwig, K. and Frankl, R. (2000). Foraging habitats and foraging distances of bumblebees, *Bombus* spp. (Hym., Apidae), in an agricultural landscape. *Journal of Applied Entomology*, **124**, 299–306.
132. Waser, N.M. (1986). Flower constancy: definition, cause, and measurement. *American Naturalist*, **127**, 593–603.
133. Weiss, M.R. (1991). Floral colour changes as cues for pollinators. *Nature*, **354**, 227–9.
134. Weiss, M.R. (1995). Associative colour learning in a nymphalid butterfly. *Ecological Entomology*, **20**, 298–301.
135. Weiss, M.R. and Lamont, B.B. (1997). Floral color change and insect pollination: a dynamic relationship. *Israel Journal of Plant Sciences*, **45**, 185–99.
136. Weiss, M.R. and Papaj, D.R. (2003). Colour learning in two behavioural contexts: how much can a butterfly keep in mind? *Animal Behaviour*, **65**, 425–34.
137. Williams, I.H. (1994). The dependence of crop production within the European Union on pollination by honey bees. *Agricultural Zoology Reviews*, **6**, 229–57.
138. Wolf, T.J., Ellington, C.P., and Begley, I.S. (1999). Foraging costs in bumblebees: field conditions cause large individual differences. *Insectes Sociaux*, **46**, 291–5.

CHAPTER 13

Insects and plants: how to apply our knowledge

Human interest in the insect–plant relationship is by no means impartial. We wish to redress balances that are intrinsic to the system irrespective of whether the system is left to itself or, as is more often actually the case, is deranged by our actions. The basic problem is that we wish to perpetuate a stable imbalance in favour of certain plants (our crops) in a biological environment that is not static but in fluctuation. The previous chapters have armed us with some insights that could help us to practise agriculture in a sustainable and ecologically sound way.

Preharvest losses of agricultural crop production are between 10% and 100% when no insecticides are used. In systems based on the use of insecticides and non-chemical control methods, losses to insect herbivory come to an estimated 13%,[99] whereas in natural ecosystems roughly 10% of all annually produced plant biomass is lost to herbivory (see Chapter 2).

Alarmingly, losses to animal pests, as viewed over a period of 25 years, are increasing for several major food crops (Fig. 13.1), although the losses are more than compensated for by increasing yields per unit of area.[87] Apparently there is a positive correlation between yields of crop plants and their susceptibility to insect pests and other biotic and abiotic constraints.

When attempting to increase agricultural production in order to feed a world population with a

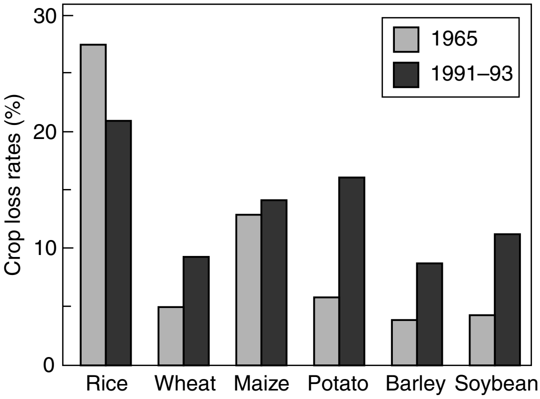


Figure 13.1 Crop losses due to animal pests for six principal food crops in 1965 and in 1991–93. (Data from Oerke *et al.*, 1994; Oerke and Dehne, 1997.)[87,88]

present growth rate of 1.2% per year, to reduce the use of synthetic insecticides, and to convert current agriculture into more sustainable systems, insights gained from insect–plant studies are indispensable. This chapter discusses aspects of insect–plant interactions that, first, may clarify why some insect species develop the status of a pest species and what measures can be taken to suppress such development and, second, may enable the control of weeds by herbivorous insects.

13.1 Which herbivorous insect species become pests and why?

13.1.1 Characteristics of herbivorous pest species

Some insect species are predestined to become pests when a favourable crop-plant species becomes available, whereas others, including closely related species, are unable to switch easily to the new food resource. Several physiological and behavioural characteristics of a species, such as fecundity, larval diet breadth, and voltinism, contribute to the likelihood of an insect attaining pest status when a suitable habitat is made available to it. An analysis of these factors has been made for some insect groups. From the biological characteristics of several *Pieris* species it can be understood why, of perhaps several dozen species or geographical subspecies of crucifer-feeding pierid butterflies, only two species (*Pieris brassicae* and *P. rapae*) have attained economic pest status on crucifer crops worldwide. Both *Pieris* species exploit a wide range of crucifers relative to other *Pieris* species and this 'euryphagy' seems to be associated with preadaptation to crop hosts. The multivoltinism of both species is another property that enables them rapidly to expand populations and to produce numerous offspring that may colonize new habitats (*Pieris rapae* females may produce more than 800 eggs). A third trait that contributes to the two species reaching pest status is a preference for dense host populations in mesic (neither extremely dry nor extremely wet) habitats.[23] Thus, several factors in combination may render an insect species a potential pest if exposed to a crop plant species that is physiologically and behaviourally an acceptable host.

13.1.2 Consequences of crop-plant introductions

Most insect–plant relationships in natural ecosystems are based on millions of years of evolution (see Chapter 11). As a result, a balance between plants and herbivores has evolved to the extent that plants are rarely eliminated solely because of insect attack. When a plant is confronted with an 'unknown' insect species the situation may be different. As Southwood noted[116] in a discussion on the evolutionary perspectives on insect–plant relationships: 'Even today when a phytophagous species first attacks a new host it often inflicts disproportionately heavy damage.' Many crop plants, especially those in the temperate zones, are introduced species. In most instances their insect pests have moved from feeding on native vegetation to feeding also on the new crop. They invade a new niche in which food is abundant and natural enemies are scarce. Moreover, the resistance of such introduced crop plants is unadapted to local insect species. An example is the potato (*Solanum tuberosum*), which originated in South America and was introduced to North America. The Colorado potato beetle *Leptinotarsa decemlineata*, living on a native *Solanum* species (*S. rostratum*), has since colonized the new food resource very successfully, because the potato did not possess constitutive resistance traits against this insect. The beetle then became a serious pest and eventually spread, with the potato, to Europe.

In fact, the number of pestiferous insect species in agricultural crops is remarkably small in view of the enormous pool of potential invaders. On a world scale, about 9000 insect species may attack agricultural crops, but less than 5% are considered to be serious pests. Whereas the total pest spectrum of a crop throughout its entire cultivation range is often very large (for instance, cacao has 1400 species, cotton 1360, sugarcane 1300) the vast majority of species actually damaging the crop are economically quite insignificant. In any crop, in one location, at one time, there is usually only a rather small number, say four to eight, of major pests in the complex that require controlling. Thus, any cotton crop, despite its huge pest spectrum worldwide, often faces in a particular region only about five insect species requiring population control.[52]

Because of the high dispersal capacities of insects, plants in natural as well as agricultural communities are normally visited by many herbivorous species. However, only a small fraction of those visitors appear to establish an enduring association with these plants. For example, only about 40 insect species have colonized soybean fields in Illinois (USA), whereas over a period of 12 years more than 400 herbivorous species were sampled in such fields. Although more than 60 aphid species were trapped in Illinois soybean fields alone, not a single aphid species has been capable of exploiting soybean as a permanent host in either North or South America.[63] The adoption of a new food plant, in this case a crop species, even if it is readily accessible in large numbers, is apparently a difficult step for most insects.

Where do insect pests come from? Do they belong to the native fauna or are they immigrants? Of 148 major insect species that infest crop plants in the USA, only 57 (i.e. less than 40%) are foreign-introduced species. Likewise, of 70 major insect-pest species in American forests the majority are native species, with less than 30% originating from Europe or elsewhere.[98] Similarly, in Europe only approximately 20% of insect pests were introduced.[97] Thus, in managed as well as natural ecosystems the majority of insect pest species are native species, although some of the most serious insect pests in forests are introduced species.

13.1.3 Agricultural practices promote the occurrence of pest problems

For reasons of mechanization and efficiency of sowing, planting management, harvesting, and processing, agricultural crops are grown predominantly as monocultures, especially in the Western world. Monocultures present favourable habitats for some insect species that thrive once food is unlimited. Why are such sytems more prone to insect population outbreaks than so-called natural systems?[103] There is no simple answer to this question, because each species or biotype of insect, each species or variety of host plant, each soil type on which they are grown, and each microclimate constitutes a specific situation. Because of the multidimensional nature of each of these components of an agroecosystem, factors that cause pest outbreaks can be diverse. Nevertheless, some of the most important factors promoting insect outbreaks in agroecosystems are obvious. They are, on the one hand, the reduced chemical and physical resistance of crop plants compared with those of their ancestral forms or closely related wild species and, on the other, the 'simplification' of the species structure of agroecosystems compared with natural ecosystems. This simplification includes a drastic reduction in plant and animal species, increased genetic uniformity of the crop, the abandonment of crop rotation, and the decrease in landscape diversity by removal of hedges, ditches, and other non-crop habitats.[103] Landscape complexity varies with the areas of uncultivated and perennial habitats such as fallows, field margins, grasslands, and woods. In structurally complex landscapes natural enemies often show increased densities compared with structurally poor landscapes.[37] An example is presented by the amount of bud damage in oilseed rape (*Brassica napus*) caused by rape pollen beetles (*Meligethes aeneus*). The extent of damage was correlated with landscape heterogeneity. Crop damage was lower and parasitism of the herbivore was higher in complex landscapes compared with simple landscapes with a high percentage of agricultural use.[124]

Some of our knowledge about host-plant resistance and the significance of polycultures for increasing agricultural diversity is discussed below. Other factors thought to stimulate the development of insect pests are discussed in detail in some recent reviews.[12,50,62]

13.2 Host-plant resistance

In nature, host-plant resistance and natural enemies are the two dominant factors controlling herbivorous insect populations. Therefore, modern approaches of pest control consider host-plant resistance breeding as a key method of insect pest regulation in crop plants. Since the beginning of agriculture, probably more than 10 000 years ago, crop plants have been selected for high yields and nutritional value, together with low mammalian toxicity and reasonable resistance against pests and disease. In our continuous efforts to develop higher yielding cultivars, very few cultivated species have retained the insect resistance level of their wild progenitors. Concomitantly, the defensive diversity is often reduced as well.[97]

Plant resistance breeding is a twentieth-century activity that stems from the knowledge of basic genetics and from the methodology of selecting, crossing, and hybridizing plants. It was undertaken fervently and became more rigorous in its approach only after the rediscovery of Mendel's laws of heredity in 1900 by Hugo de Vries. Although development of insect-resistant crop cultivars using classical breeding methods is a time-consuming and expensive process, the benefits may be enormous in terms of monetary return and reduced burdening of the environment with insecticides. The economic advantage of using pest-resistant cultivars is estimated to be a 120-fold greater return on investment and, no less importantly, some new cultivars of cotton, rice, and vegetables developed recently contain insect resistance sufficient to eliminate the use of insecticides entirely.[72,114] There is an abundant literature documenting the genetic control of arthropod resistance and resistance variation in agricultural crops, as evidenced by extensive reviews by Maxwell and Jennings,[75], Fritzsche *et al.*,[41] and Panda and Khush,[90] and a bibliography by Stoner.[117] From these studies it can be concluded that the reason why resistant crop varieties are so rare is not because of a lack of resistant resources. It is the complicated and undesirable large-scale insect bioassays that make breeders reluctant to incorporate resistance to (mobile) insects in their breeding programmes. Therefore, new technologies such as genetic modification and molecular marker-assisted selection (MAS),[70] which avoid insect bioassays, are highly valued for this particular purpose.

13.2.1 Host-plant resistance mechanisms

For a long time researchers in host-plant resistance breeding have been concerned mostly with methods of rapidly identifying resistant genotypes in germplasm banks and monitoring the inheritance of resistance in breeding lines, while being less interested in the mechanisms underlying resistance. A scientific basis of the field and a more systematic research approach was introduced by Painter,[89] and in recent overviews plant-breeding methodology and analysis of resistance mechanisms have been integrated.[90,111,135]

Painter recognized three 'causes' of resistance, emphasizing those aspects of insect–plant relations that are relevant to insect resistance: (1) non-preference, (2) antibiosis, and (3) tolerance. *Non-preference* defines the group of plant characters and insect responses that lead away from the use of a particular plant variety for oviposition, for food, for shelter, or for combinations of the three. Because the term 'non-preference' describes the response of the insect rather than a plant characteristic, it has been replaced by *antixenosis* (*xenosis* is Greek for 'guest'; antixenosis thus means 'against guests'), defined as plant properties evoking negative (non-preference) responses or total avoidance by insects.[64] *Antibiosis* denotes reduced fecundity, size, or longevity, and increased mortality of the attacking insect. Antibiosis, in contrast to antixenosis, clearly refers to those plant properties that adversely affect the physiology of a herbivore. *Tolerance* is a form of resistance in which the plant shows an ability to grow and reproduce or to repair injury to a marked degree in spite of supporting a herbivore population approximately equal to that damaging a susceptible host. Tolerance is a plant property that is expressed irrespective of whether an insect (or another organism) is responsible for tissue loss. Unlike antixenosis and antibiosis, it does not represent a selection pressure on herbivore populations. Therefore, present-day convention no longer considers tolerance as a subcategory of 'resistance', but puts it as a plant defence mechanism next to resistance.[118] When the pest insect is a vector for one or more plant pathogens, tolerance is an undesirable trait, as the insect population may increase on the crop, enhancing the risk of pathogen spreading.

Whereas antixenosis and antibiosis lend themselves well to deliberate selection in specific laboratory and standardized field tests, tolerance is a modality of plants that is more difficult to assess because it requires simultaneous observation of insect populations and yield potential of adult plants.[135]

So far, tolerance seems to be the least common type of defence mechanisms. An examination of more than 200 reports on resistance to arthropod pests in vegetables showed that tolerance was involved in about 10%, whereas the remaining cases were equally attributed to either antixenosis or antibiosis.[117] However, tolerance has been documented to occur in at least 13 crop species[36] and its lower reported frequency may also reflect the relative attention paid to it.

It must be emphasized that, although the triad classification has proved to be a very useful one, resistance to insect attack is most frequently a combination of two or even all three types of defence mechanism.

13.2.2 Partial resistance

Although some examples exist, it is often difficult to attain complete resistance to a particular insect species, and only partial resistance can be obtained. An advantage of incomplete resistance is, however, that it poses weaker selection pressure on the insect population and consequently is more durable. In combination with various integrated pest management measures, partial resistance may be sufficient or even preferable because of the reduced risk of the development of new virulent insect biotypes.[46,47]

In this context two more terms need to be introduced. *Horizontal* or *polygenic resistance* is a quantitative trait governed by a mixture of minor resistance genes that are accumulated in one genotype. *Vertical resistance*, conversely, is resistance governed by one or more genes in the host plant, each of which corresponds to a matching gene for parasitic ability in the pest species (it is therefore sometimes called *gene-for-gene resistance*). Numerous cases of polygenic resistance to insects are known to occur in many crop plant species. Many instances of monogenic resistance have also been reported in the literature. The most extensively studied inheritance of the latter type is that of wheat resistance to the Hessian fly, as a result of which 26 genes for resistance have been identified.[90] For every resistance gene available in wheat there are corresponding genes known ('gene-for-gene') in the fly that enable it to overcome the resistance.

There is an important difference between the two resistance types with respect to their stability. Horizontal resistance involves the accumulation of genes from diverse germplasms. Building up a satisfactory level of resistance is a time-consuming process. This is compensated for by the fact that this resistance is generally more difficult to overcome by resistance-breaking insect biotypes and thus generally more stable than vertical resistance.

Resistance stability is sometimes of short duration, particularly when the resistance level is very high, its inheritance is simple, and the resistant cultivars are grown on a large scale. Under these circumstances insects may break the resistance by developing biotypes that possess an inherent genetic capability to overcome host-plant resistance. Cases are known in which as few as three generations were required to select resistance-breaking biotypes, and occasionally the insect's potential to overcome plant resistance is so great that the effect of the resistance is nullified before the resistant cultivar reaches widespread use. This happened, for instance, to cultivars of Brussels sprouts resistant to the cabbage aphid *Brevicoryne brassicae*.[34] Several strategies exist to improve the durability of resistance, for instance by reducing selection pressure on the pest species.[35] Usually, however, adaptation to new cultivars takes longer, even under strong selection regimes,[39] and several examples of long-lasting resistance are known. The apple variety 'Winter Majetin', for instance, which was reported to be resistant to the woolly apple aphid *Eriosoma lanigerum* as long ago as 1831, still retains this trait. Another often-cited example is the partial resistance of grape vines to the grape phylloxera aphid *Phylloxera vitifoliae* in French vineyards, which has been effective since 1890.[90]

Polygenic resistance is probably not *per se* more durable (for instance, not when it involves the concentration of a single chemical compound), but when it relates to multiple chemical, physiological, or morphological mechanisms the chances that a pest species will break resistance are much lower. When trying to understand why in some cases resistance is easily overcome whereas in others it is durable, the insect's adaptibility is a critical factor. Conceivably, insects may adapt physiologically to the presence of, for instance, toxic compounds in their food more easily than they can adjust behaviourally to new plant characteristics. In the latter case a series of changes is needed, including adaptation to various cues governing oviposition behaviour and feeding. This view agrees with the fact that plant breeders selecting for insect-resistant genotypes consider the antixenotic type of resistance more valuable than the antibiotic type. This is because in their experience the latter type is generally less durable.[117]

13.2.3 Plant characteristics associated with resistance

Not surprisingly, plant features causing resistance in cultivated plants do not differ from those operative in wild plant species—physical (Fig. 13.2), chemical, or phenological factors. Many such factors have been identified, and numerous examples are given elsewhere in this book. For information on resistance mechanisms identified in specific crop-plant species, reviews of the extensive literature should be consulted.[75,90,114,117]

Resistance based on morphological traits often provides long-lasting protection, compared with most chemically based resistance. Still, physical barriers are not unsurmountable, as evidenced by the beetle *Jadera haematoloma*, which feeds on the seeds of sapindaceous tree species in North America. This herbivore has evolved different beak lengths in response to the introduction of new hosts within only 50 years, enabling it to feed on larger or smaller seeds than those of its original host (Fig. 13.3).[22]

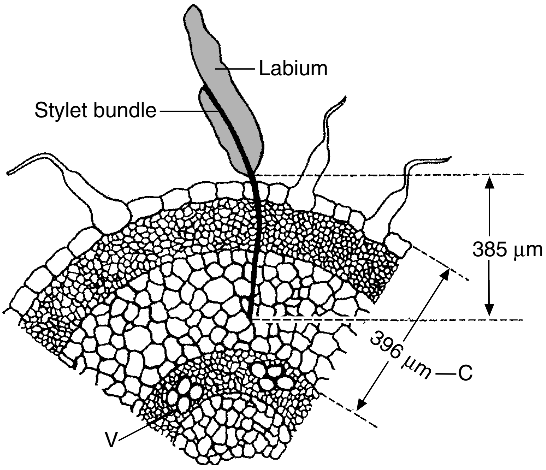


Figure 13.2 Insect resistance caused by plant anatomical characteristics. A thick cortex (C) in stems of *Lycopersicon hirsutum* prevents aphids (*Macrosiphum euphorbiae*) from reaching vascular tissue (V) with their stylet bundles. (From Quiros *et al.*, 1977.)[100]

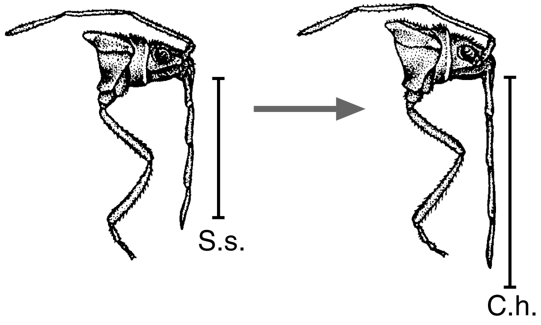


Figure 13.3 The seed-feeding bug *Jadera haematoloma* is specialized on members of the plant family Sapindaceae. With its slender tubular beak the insect reaches through the walls of sapindaceous fruits to the seed, pierces the seed coat, and sucks its liquefied contents up. Populations living on a native tree *Sapindus saponaria* (fruit radius: 6.1 mm) have beak lengths of 6.7 mm, whereas populations on the recently introduced species *Cardiospermum halicacabum* (fruit radius: 8.5 mm) show beak lengths of 7.8 mm. S.s. and C.h., radii of host fruit drawn to scale. (From Carroll and Dingle, 1996.[22] © 1996, with permission from Elsevier.)

Usually, the incorporation of resistance genes into high-yield cultivars requires some sacrifice in yield and can therefore be considered to impose a 'cost'. This is due to the fact that most forms of plant resistance appear to involve some diversion of resources by the plant to increased production of allelochemicals or extra physical defence structures. Under natural conditions defence systems are maintained under the selection pressure of a plant's enemies, but they are loosened in the absence of herbivores in order to save unnecessary 'costs' of defence. This hypothesis is supported by the outcome of the following experiment. A field population of *Arabidopsis thaliana* was protected against insects and pathogens, and resulted in an alteration of the pattern of selection in two characters shown to reduce herbivore damage: total glucosinolate concentration and trichome density.[74] This observation clearly shows that the plant species tries to save on defence costs when the risks of attack are reduced.

In the past, selection of crop species for improved agricultural value has been associated with reduced levels of particular secondary plant substances (Table 13.1) and, as a consequence, increased herbivore susceptibility. The observation that the best soybean breeding lines resistant to various insect herbivores still yield less than the best available cultivars grown in the absence of pests[63] fits the assumptions made above.

Likewise, when, under insect-free conditions, two barley cultivars with an isogenic difference in greenbug (*Schizaphis graminum*) resistance were grown in competition, the susceptible cultivar was the better competitor. However, when the cultures were exposed to aphid feeding the outcome was reversed: the resistant cultivar became the better competitor.[139] In other cases such costs appear more difficult to detect. One of the causes of contradictory results on the costs of resistance is that the relative performance of resistant and susceptible genotypes depends on several environmental conditions that may easily mask the costs involved in resistance traits.[13]

Table 13.1 Effect of domestication on amounts of secondary metabolites

Compounds	Plant	% of wild species	Reference
Quinolizidine alkaloids	Lupin	0.5	140
Cucurbitacins	Squash	1	55
2-Tridecanone	Tomato	1.5	137
Glycoalkaloids	Potato tubers	4	59
Glucosinolates	Cabbage	20	60
Gramine	Barley	20	71

Concentrations of secondary compounds in crop plants are expressed as a percentage of those in wild relatives.

As different insects have different nutritional requirements and show different responses to plant defence factors, a particular plant cultivar selected for resistance to one insect species usually remains susceptible to other insect species. Multiple species resistance is often difficult to develop. This is illustrated by the difficulties encountered when developing resistance to three major pest species of cotton. Whereas smooth-leaf cultivars suffer less from the larvae of some *Helicoverpa* species, they are prone to increased feeding damage from tarnished plant bugs (*Lygus lineolaris*). Frago-bract strains, in which the bracts are modified such that the cotton buds are exposed, show reduced infestation by boll weevils (*Anthonomus grandis*), but increased susceptibility to tarnished plant bugs. Despite these obstacles it has been possible to develop cotton cultivars that exhibit resistance to all three insects, as well as to cotton leaf-hoppers (*Pseudatomoscelis seriatus*).[16] This shows that satisfactory protection may be attained even to insect pest complexes.

Plant resistance against herbivores has not only a 'direct' but also an 'indirect' component, through an influence on the third trophic level. Different cultivars may differ in the production of entomophage-attracting allelochemicals as, among others, has been observed in gerbera plants (*Gerbera jamesonii*) for predatory mites.[69] If this aspect is neglected in a plant-breeding programme, such an indirect resistance factor may inadvertently be eliminated. This would result in the selection of cultivars with a reduced net resistance under field conditions, especially where natural enemies play a significant role in herbivore mortality.[31]

13.2.4 Methodology of resistance breeding

Plant breeding exploits genetic variability within the crop species and its wild relatives, and aims to enhance resistance to insect pests and diseases by prudent selection and breeding methods. Present-day approaches involve the combination of (1) the use of population growth models for exploring resistance management strategies, (2) developing efficient test procedures, (3) a further exploitation of antixenosis as a resistance modality, and (4) evaluating the potential of molecular biological techniques.[109]

Conventional methods of insect resistance breeding

As sources of resistance genes there are a number of broad-based germplasm collections, consisting of wild species as well as large numbers of cultivars, located in different parts of the world.[36,113] More than 1300 collections that are registered in the World Information and Early Warning System (WIEWS) database of the Food and Agriculture Organization of the United Nations (FAO) stock numerous accessions, containing valuable gene pools for crop improvement activities. However, the observation that 80–90% of the varieties of several important food crops have been lost during the past 100 years indicates a truly alarming impoverishment of our potential food reservoir.[129]

Wild relatives provide valuable source materials for insect and disease resistance.[2] For instance, high levels of resistance to two plant-hopper species have been transferred from *Oryza officinalis* to cultivated rice (*O. sativa*).[57] It is estimated that plant breeders nowadays still return to land races and their wild relatives for about 6% of the germplasm lines used in their breeding programmes.

As mentioned above, insect resistance sometimes depends on one locus (monogenic), but more often several independent loci (oligogenic) or even many loci (polygenic) are involved that confer resistance in different ways. Traits with simple mendelian inheritance are relatively easy to work with. Monogenic resistance has frequently been found in crop plants. A classical example is resistance to the brown plant-hopper *Nilaparvata lugens* of rice.[90] In wild plants, however, resistance to an insect is seldom based upon a single resistance gene. Several modes of defence (e.g. chemical, physical, and imbalance of nutritional factors) are combined and controlled by a complex system with several loci and multiple alleles at one locus. When resistance genes are located in exotic germplasm, much work is required to incorporate them into more agronomically acceptable lines. Depending on the reproductive system of the crop species (i.e. self-pollinating or cross-pollinating), various breeding programmes can be used, as described in books on the methodology of breeding for insect resistance, such as those by Panda and Khush[90] and Smith *et al.*[115]

Biotechnology

Traditional selective breeding can now in some cases be short-circuited by ingenious biotechnological methods. Recent advances in molecular biology and tissue culture have made it possible to transfer genes not only from related species but also from unrelated plants and other still more distantly related sources, such as animals, bacteria, and viruses.[8,109] Genetic engineering methods permit the introduction of novel genes into crop species that render them resistant to insects. For instance, genes from insect pathogens introduced into the insect's food plant may result in effective insect population control. Thus, genes responsible for the production of a toxin derived from the insect pathogen *Bacillus thuringiensis* have been introduced into, among others, tomato, rice, cotton, and spruce trees. Other orally active adverse proteins, such as lectins, amylase inhibitors, and proteinase inhibitors, which retard growth and slow down development, have also been produced in transgenically modified plants. For instance, by transferring cDNA that encodes the α-amylase inhibitor occurring in the seeds of *Phaseolus vulgaris* into pea (*Pisum sativum*), resistance to the pea weevil *Bruchus pisorum* was conferred. Transgenic pea seeds accumulated the α-amylase inhibitor to a level of 3% of soluble protein. The inhibitory effect on human α-amylase should disappear through cooking.[108]

The introduction of transgenic crops is taking place at a spectacular rate. The area occupied by four commercialized transgenic crops—soybean, maize, cotton, and oilseed rape—has within 9 years increased to 29% of the total global area covered by these crops (Fig. 13.4). Herbicide tolerance is the dominant trait (72%), followed by insect resistance (20%). The introduction of stacked genes (i.e. the insertion of two or more major genes in one

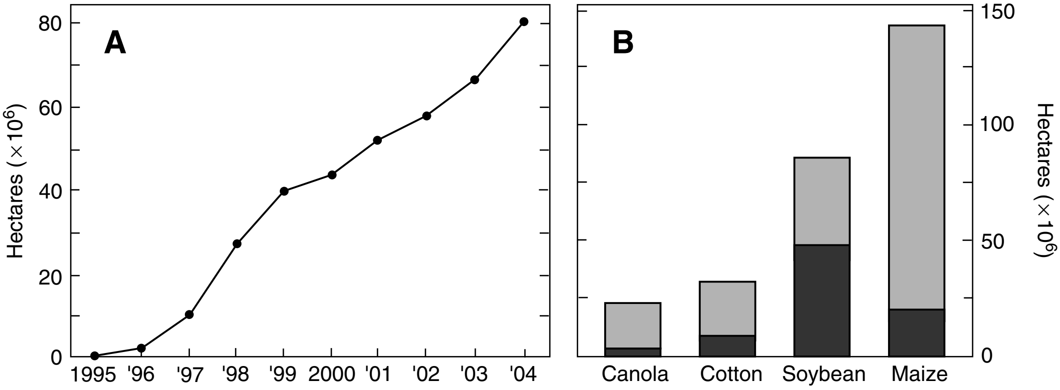


Figure 13.4 (**A**) Global area planted with genetically modified crops from1996 to 2004. (**B**) Global areas of four crops (2004). For each crop species the hectarage occupied by commercialized transgenic plants is indicated in black. (Data from James, 2004.)[56]

cultivar) for herbicide tolerance and insect resistance has started more recently. This combination is deployed in both cotton and maize, and by 2004 already occupied 7% of the global area covered by both transgenic crops.[56]

Although the transgenic approach can be considered as a specific technique to obtain host-plant resistance, there are a few important differences with conventional plant breeding, apart from the fact that in transgenic crops a gene from another organism is incorporated into the genome of the plant. The gene that is incorporated into the genome is usually known in great detail, as is its product. In conventional plant breeding closely related plant species or cultivars are crossed, and it is largely unknown which genes and corresponding phenotypic traits are responsible for the enhanced host-plant resistance. When plant characteristics responsible for increased insect resistance are unknown, it is difficult to determine their environmental impact and their effects on the third trophic level.

Another difference between conventional plant breeding and, for instance, *Bt* crops (i.e. crops containing *Bacillus thuringiensis* genes coding for insecticidal proteins) is the number of genes that are involved in plant resistance. Stability and durability of host-plant resistance depends to a large extent on the genetic basis of resistance in plants, that is, whether it is due to one major or many minor genes. *Bt* crops developed so far are resistant to insect damage as a result of one gene that is incorporated into the plant genome, and can thus be considered as a form of vertical resistance. Vertical resistance is generally less stable than horizontal resistance, because it can be overcome by certain biotypes of insect.[90,113]

There are several options to improve the durability of vertical resistance:[113]

(1) sequential cultivar release—the release of one major gene that is used until it becomes ineffective, after which additional genes are released successively. This method has been used for the deployment of genes in rice with brown planthopper resistance;
(2) gene pyramiding or stacking. For example, rice cultivars currently in use have resistance based on six genes that were formerly used in sequential cultivar release;
(3) gene rotation, in which one gene is alternated with another gene.

In the case of transgenic *Bt* crops sequential cultivar release is undesirable, as resistance developed to one toxin may confer cross-resistance to related toxins.[120] In addition, for organic farmers who use *Bt* sprays such a development would be detrimental for insect control.

A third major difference between conventional plant breeding and transgenic *Bt* crops is the expression level of the insect-resistant plant characteristics in the newly developed lines. In conventional plant breeding the expression level of a characteristic, for example a plant allelochemical, can never become higher than the maximum occurring in nature. In *Bt* crops the expression level of the gene coding for the *Bt* toxin is determined by the (modified) promoter,[68,92,93] and the number of gene copies inserted into the plant genome.[65] In this way the level of *Bt* toxins in transgenic plants can become as high as 2–5% of the total protein level in plants, which is 20 to 50 times greater than in the current transgenic *Bt* plants. The creation of plants with expression levels as high as possible may not only have a much higher impact on non-target organisms than the doses studied and used so far, but may also increase the chance of resistance development in target insects. The pros and cons of *Bt* toxin technology have been reviewed recently by Haq and co-workers.[49]

Whether transgenic insect-resistant crops will be a valuable contribution to pest control that is more environmentally friendly will depend on several aspects. First, the effects of the transgenic characteristic on non-target organisms, including pollinators, biological control agents, and protected rare species are important. This relates to both above-ground and below-ground interactions.[48,67] Second, the durability of the resistance in terms of the development of adaptation by pest insects will affect whether or not a transgenic line will be a short-term or a long-term solution.[121] Finally, outcrossing of the transgene and introgression into wild relatives is an important aspect to consider, because once the transgene has moved to wild relatives there will be no way back and the characteristic may then affect ecological interactions in non-agricultural ecosystems.[29] The rapidly expanding literature about potential ecological risks (and benefits) of transgenes has been clearly outlined in reviews by Pilson and Prendeville[95] and O'Callaghan *et al*.[86]

Obviously, genetic engineering opens fascinating avenues for crop improvement[49,76,77] and insect resistance, and has been designated by some as the ultimate technique in agricultural production. In comparison to traditional insecticide applications, the elegant technique of built-in insect toxicity provides an obvious improvement, because toxic effects on non-target species are reduced. However, resistance based on a single strong toxin has disadvantages compared with other resistance mechanisms. It is well documented that insects develop resistance against particular insecticides and this problem may similarly arise in genetically modified crops owing to the development of resistant insect biotypes. One of the best strategies of resistance management is the so-called high-dose–refuge strategy. This procedure aims at reducing the risk of resistance development in the pest insect by the mandatory planting of refuges of toxin-free crops near *Bt* crops to promote survival of susceptible pests.[21]

Thus, valuable though genetic modification methods may be, lessons from the past strike a serious note of caution.[48] As Stoner rightly stated:[117]

> It is much too soon to abandon traditional approaches to plant resistance to insects. Researchers in the field of plant resistance to insects should take advantage of the opportunities presented by new developments in biotechnology, but should also maintain their unique focus on the behavioural, physiological, ecological, and evolutionary interactions of the insect with its host plant. (p. 137)

13.3 Polycultures: why fewer pests?

From time immemorial, farmers have known that growing several crops on one unit of land resulted in increased yields. Pliny the Younger (23–79 AD) wrote in his *Naturalis Historiae* that when oilseed rape (*Brassica napus*) and common vetch (*Vicia sativa*) were grown together many insects normally occurring on these crops remained absent. Since then, numerous studies have evaluated the impact of plant mixtures on insect population dynamics.[3,54] One study on flea beetle (*Phyllotreta cruciferae*) infestation of different broccoli/*Vicia* spp. planting systems may be cited to illustrate Plinius' obervation. Figure 13.5 depicts the flux of marked flea beetles from diculture broccoli/*Vicia* plots to monoculture broccoli plots and surrounding habitats. After vacuuming all naturally occurring flea beetles, three groups of 350 flea beetles, each coloured differently, were relased in each plot. During a 24-h period after release, high migration rates of beetles out of the diculture plots and between the monoculture plots and surrounding habitats were observed. In particular, more beetles

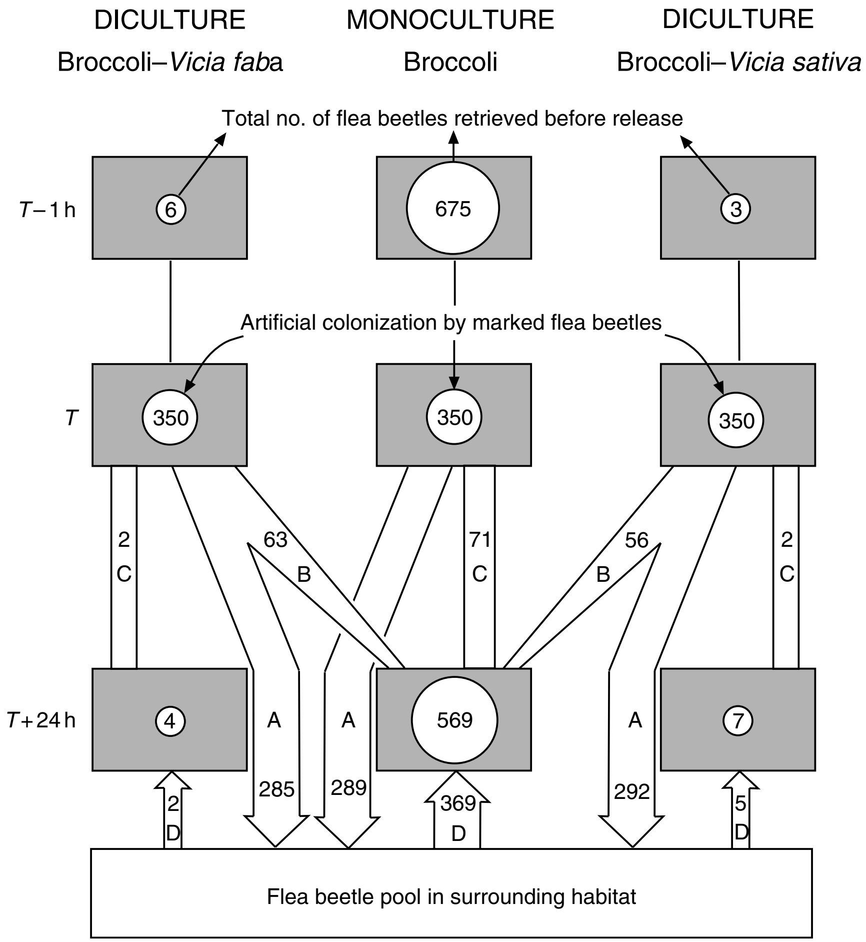


Figure 13.5 Flux of marked flea beetles (*Phyllotreta cruciferae*) in three experimental plots with different cropping systems during a 24-h period after release. After the plots were cleared of all naturally occurring flea beetles, three groups of 350 flea beetles marked with fluorescent blue, orange, or pink were released in each plot at time *T*. Fluxes are indicated by: A = beetles emigrating out of crop habitat; B = beetles moving from dicultures to monoculture; C = beetles staying in the plots; D = beetles colonizing plots from surrounding habitats. (Redrawn from Garcia and Altieri, 1992,[42] with kind permission of Springer Science and Business Media.)

left the mixed cultures than the monoculture, resulting in faster reduction of artificially introduced flea beetle populations in the mixed systems.[42]

Intercropping is still common in subsistence agriculture in the tropics, where the percentage of cropped land devoted to polycultures varies from a low of 17% in India to a high of 94% in Malawi.[131] By contrast, modern intensive agriculture in the Western world has reached a shockingly high degree of bio-uniformity. Large acreages are planted with monocultures of only one out of a few cultivars, which often possess very low genetic diversity. Increasing vegetational diversity by planting different crops intermingled is one type of cultural control strategy that can make agro-ecosystems less favourable to the pest insect and/or more favourable to natural enemies.

Terms related to polycultural planting schemes are sometimes used rather loosely and inconsistently. *Intercropping* describes a system whereby more than one crop is grown in an area simultaneously, in such a way that the crops interact agronomically. Intercrops can be of four types:

(1) *mixed cropping*—growing two or more crops simultaneously with no distinct row arrangement;
(2) *row intercropping*—one or more of the crops grown simultaneously in different rows;
(3) *strip intercropping*—two or more crops are grown in strips wide enough to permit independent cultivation, but narrow enough for the crops to interact agronomically;
(4) *trap-cropping systems*—one species serves as a trap crop to decoy the pest away from the major crop.

Intercropping does not necessarily involve two different plant species. It can also be practised at two other levels of uniformity: the variety and the gene level.

Multiple cropping refers either to intercropping (i.e. crops growing simultaneously) or to sequential cropping (i.e. growing two or more crops in sequence on the same field per year).[131]

Interactions between component crops make intercropping systems more complex and at the same time frequently reduce pest attack. Overwhelming evidence suggests that polycultures support a lower herbivore load than monocultures. A survey of 209 published studies on the effects of vegetation diversity in agro-ecosystems on herbivorous arthropod species showed that 52% of the total herbivore species were found to be less abundant in polycultures than in monocultures, whereas only 15% of the herbivore species exhibited higher population densities in polyculture (Table 13.2).[5] A meta-analysis of 21 studies showed that in 60–70% of cases herbivore densities were lower in diversified treatments than in monocultures. It was concluded that crop diversification has only a moderate effect on the abundance of herbivorous insects.[127]

As might be predicted, cases of lower abundance in polycultures were predominantly among the food specialists. In contrast, polyphagous species often (though not always[14]) fared better and exhibited higher densities in polycultures (Table 13.2). Of course, not all combinations of crops are equally effective in this respect, and the choice of the partner crop is more important than the simple decision to practise intercropping. Combining wheat and maize, for instance, would actually increase the damage level inflicted by shared pests, such as chinch bugs (*Blissus* spp.) and nematodes, whereas intercropping wheat with potatoes would reduce the damage to wheat.

Although many studies have documented differences in single versus multicropping systems in terms of intensity of herbivore attack, precise information is lacking on the mechanisms that generate these effects. Numerous biotic and abiotic factors vary between the two practices, including plant density and structural complexity, microclimatic factors such as temperature, shadiness, and humidity, refuges, alternative food sources for natural enemies (flowers, extrafloral nectaries), masking and repellent odours, and camouflage.[11] Yet, the discovery of underlying mechanisms of yield responses to intercropping is vital both for generating predictive theory and for the application of this knowledge in managed systems.

Three theories that attempt to explain reduced pest infestations in polycultures have received much attention: (1) the disruptive-crop hypothesis;

Table 13.2 Relative abundance of arthropod species in polycultures compared with monocultures (from Andow, 1991)[5]

	% more abundant	% no difference	% less abundant	% variable	Total no. of species
Herbivores					
Monophagous species	8	14	59	19	220
Polyphagous species	40	8	28	24	67
Natural enemies	53	13	9	26	130

Values are percentages of total numbers of species. A variable response means that an arthropod species did not consistently have a higher or lower population density in polyculture compared with monoculture when the species response was studied several times.

(2) the enemies hypothesis, and (3) the trap-crop hypothesis.[3,131]

13.3.1 The disruptive-crop hypothesis

A basic observation in ecology is that consumers tend to concentrate at places where their resources are abundant and easy to find. Root[105] formalized this phenomenon as the 'resource concentration hypothesis' (see Section 10.8). The hypothesis predicts that herbivores are more likely to find and remain on host individuals grown in monoculture than host plants grown in spatially diluted systems (i.e. polycultures). Not only may insect populations be influenced directly by the spatial dispersion of their host plants, there can be also a direct effect of associated plant species on the ability of the insect herbivore to find and utilize its host. Volatiles emitted by non-host intercrops may mask the odour of the host plant, thereby disrupting host-finding behaviour of the pest insect. Such 'olfactory masking' has been shown, for example, in relation to the orientation of Colorado potato beetles to potato odours. In laboratory experiments, starved Colorado potato beetles exhibit strong positive anemotactic responses to air currents blown over potato foliage, whereas responses to air streams with tomato odours do not differ from those to clean air. The attractiveness of host-plant odour, however, is completely masked in a mixed odour blend of the two plant species (see Fig. 6.13).[123] A well known example of olfactory masking is the old practice of interplanting carrots with onions to prevent attack by carrot flies.[130] Several aromatic herbs, likewise, have been used to repel insects infesting vegetable crops. Brussels sprouts intercropped with the herbs sage (*Salvia officinalis*) and thyme (*Thymus vulgaris*) received fewer eggs from the diamondback moth *Plutella xylostella* than pure stands, through an olfactory effect of the labiate herbs.[33]

Insects in polyculture also show an increased tendency to leave their host plant, often followed by migration out of the field. In the case of the striped cucumber beetle *Acalymma vittata*, densities reached in polycultures of cucumber, corn, and broccoli were 10 to 30 times lower than those in monocrops of cucumber (Fig. 13.6).[9] Interestingly, in this case the associated crops also had an indirect effect on the insect via its host plant. When under laboratory conditions the beetles were offered a choice between leaves taken from monocultures and those from cucumber plants intercropped with tomatoes, the insects preferred the foliage from plants in pure stands.[10] This indicates that plant-stand diversity and host-plant quality may interact in a complex way.

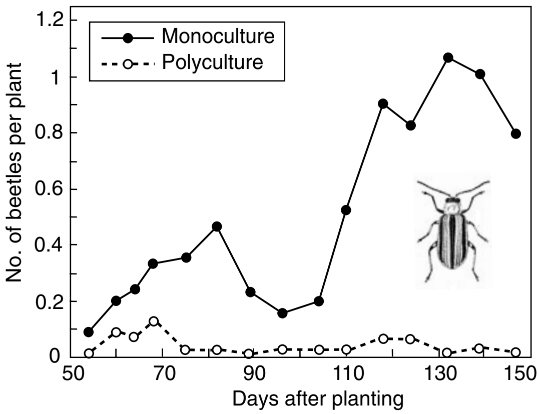


Figure 13.6 Mean number of striped cucumber beetles per plant in high-density plant systems of cucumber plants alone (monoculture) and cucumber intercropped with maize and broccoli plants (polyculture). (From Bach, 1980.)[9]

It seems likely that this type of allelopathic interaction between (undamaged) plants affecting higher trophic levels are quite common. Thus, it has been reported that barley plants show reduced acceptability to aphids after the host plants have been exposed to volatiles produced by thistle plants.[44] In laboratory tests some barley cultivars also exhibited decreased acceptability to bird cherry-oat aphids (*Rhopalosiphum padi*) after exposure to air from certain other cultivars. This finding concurs with field observations showing that aphid acceptance was changed when some combinations of barley cultivars were grown side by side in separate rows.[84] The mechanisms responsible for these allelopathic effects remain to be elucidated.

13.3.2 The enemies hypothesis

According to Root's enemies hypothesis,[105] generalist and specialist natural enemies of insect pest species are expected to be more abundant in polycultures than in monocultures, because polycultures often offer additional food sources, such as honeydew, nectar, and pollen, and more refuges where insects can shelter in the shade and encounter higher humidity during hot periods. In addition, more alternative prey or herbivore hosts may be available in periods in which the pest species is scarce.[24] Natural enemies show lower emigration rates from diverse plant assemblages, whereas immigration rates are not affected.[25] A literature survey showed that 68 (53%) of a total of 130 natural enemy species did indeed attain higher population densities in polycultures compared with monocultures, whereas in only 9% of the observed cases were lower population densities encountered (Table 13.2).[5] The dramatic yield increase of 100% for maize in a triculture with faba bean (*Vicia faba*) and squash (*Cucurbita moschata*) compared with the yield in a maize monoculture was due to reduced population densities of aphids (*Rhopalosiphum maidis*) and spider mites (*Tetranychus urticae*). In the triculture, aphids experienced higher levels of attack by several species of arthropod predator, including two ladybird species more constantly associated with aphids in the diversified system.[128] An analysis of the causes of reduced insect pest levels through polycultural practice showed that in 12 of 36 studies the effect was primarily due to natural enemy action (Table 13.3).[11]

13.3.3 Trap-cropping and crop–weed systems

Trap crops are plant stands in the vicinity or in certain parts of a field where the principal crop is grown that attract pest insects so that the target crop escapes pest infestation. Trap-cropping systems have been found to be particularly useful to subsistence farmers in tropical countries. Thus, a tomato monoculture in Central America was totally destroyed by *Spodoptera sunia* caterpillars, whereas intercropping of tomatoes with beans was effective in reducing the attack to virtually zero. The caterpillars of *S. sunia* were all attracted to the bean plants, which served as a trap crop. To date trap-cropping has played a major role in only a few crops: cotton, soybeans, potatoes, and cauliflower. Of these, the cotton and soybean trap-cropping systems clearly have the greatest importance worldwide, although plenty of successful examples suggest that this strategy could be used in more cases than it currently is.[53] Several small-scale experiments on protecting, for instance, leek, cabbage,[7] and sweet corn[102] against specialist or generalist herbivores by employing trap crops indicate that this method merits further exploration.

Table 13.3 Relative importance of regulating mechanisms in 36 reports on reduced insect pest levels in intercropping systems (from Baliddawa, 1985)[11]

Pest population controlling factor	Occurrence
Lowered resource concentration, trap-cropping, microclimate, and physical obstruction	9
Reduced colonization	5
Masking and camouflage	5
Repellency	5
Natural enemies	12

Whereas weeds can act as reservoirs of pests, and many pest outbreaks can be traced to locally abundant weeds belonging to the same family as the affected crop plants,[125] weeds often harbour a beneficial entomofauna that may affect herbivore populations on adjacent crop plants positively. Because weeds can offer important resources for natural enemies, such as alternative food and microhabitats that are not available in weed-free monocultures, certain types of crop pest are less likely to develop in weed-diversified crop systems. Many examples are known of cropping strategies in which the presence of weeds enhances the biological control of specific crop pests, ranging from fruit crops (e.g. apple) to vegetables (e.g. Brussels sprouts), fibre crops (e.g. cotton), grains (e.g. sorghum), and grapevine.[3] In an experiment to investigate 'green' pest-control methods, which abandon the use of conventional insecticides in apple orchards, selected weeds were sown in strips to attract aphidophagous predators. After some time predaceous arthropods were more abundant in the strip-sown area of the orchard than in the control weed-free area. This difference was paralleled by significantly reduced numbers of two detrimental aphid species (Fig. 13.7).[141] Likewise, plant diversification in vineyards lowered population densities of grape leaf-hoppers and thrips when compared to

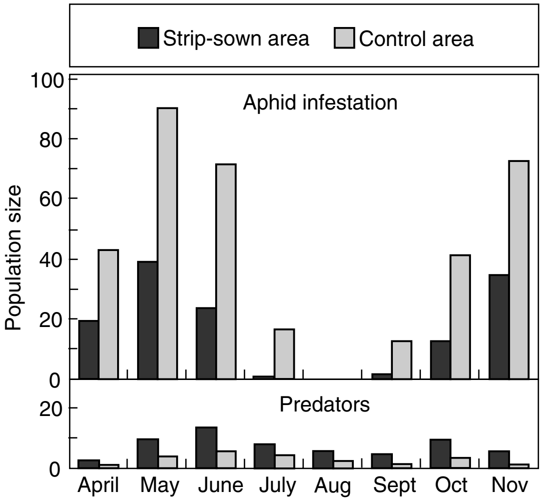


Figure 13.7 Aphid infestation rates and mean number of predaceous arthropods in an apple orchard. In one part of the orchard weed strips were sown in the existing ryegrass; the other part was used as a control area. Aphid population size was recorded in infestation classes and aphidophagous predators were recorded in absolute numbers per tree. During summer the aphids *Dysaphis plantaginea* and *Aphis pomi* live on alternative host plants. (From Wyss, 1995.)[141]

monoculture control plots. This was correlated to an increase in the abundance of natural enemies by about 50%, resulting in enhanced biological control of the two herbivorous insects.[83]

An additional method is to devote field edges to flowering weeds that supply natural enemies of herbivorous arthropods with nectar. In the selection of the flowering plants it is important, however, to determine whether pest insects can benefit from the nectar-producing plants or not. After all, many adult pest insects consume only nectar, whereas their larvae consume the crop.[85]

Field experiments involving several crops have also shown that careful diversification of the weedy component of agricultural systems often lowers pest populations significantly. More details of insect manipulation through weed management are given by Altieri and Nicholls.[3]

13.3.4 Diversity as a guiding principle

Agriculture implies the simplification of nature's biodiversity, resulting in an artificial ecosystem requiring constant human intervention. In monocultures diversity is glaringly absent. This means that there are no alternative host plants for pest insects, nor are there salubrious environments for natural enemies. Diversification is probably a key element in future insect-control strategies in agriculture,[97,119] and polycultures may provide an important step towards that future. There is an interesting form of polyculture that negates some technical disadvantages of culturing mixtures of two crop species: growing combinations of genetically different crop cultivars. When a cassava cultivar susceptible to whiteflies was grown intercropped with a cultivar that possessed partial resistance to the whitefly *Trialeurodes variabilis*, the overall population density of this insect in the intercropped system was 60% lower than that in the monoculture.[45] So far, however, the potential gains of growing mixtures of resistant and susceptible varieties of a crop plant species are still largely unexplored.

Polyculture strategy has often been found to increase yields, sometimes to a considerable extent. An analysis of the mechanism causing the reduction of a pest population is not so easy, especially as several factors are often involved. Table 13.3, although based on a limited number of studies, shows that a variety of mechanisms, including lowered resource concentration, natural enemy action, and various diversionary mechanisms, may be operating and be responsible for higher yield under polycultural practices.

13.4 Plant-derived insecticides and antifeedants

In view of the ample evidence that most, if not all, herbivorous insects are inhibited from feeding by secondary compounds in non-host plants, it is a logical step to exploit such substances for the protection of our food crops. Indeed, since the dawn of civilization humankind has used plant materials to combat insect pests or alleviate the damage they cause. Although early agricultural writings frequently contain references to the use of plant extracts for pest control, the descriptions of the plants are often so vague as to make identification impossible. Nevertheless, well documented records show that before 1850 20 plant species belonging to 16 different families were used for control of agricultural and horticultural pests in western Europe and China.[82,112] A recent review shows that a multitude of plants is traditionally being used to protect stored plant seeds against beetle pests in western Africa.[18]

A resurgent interest in the use of plant-derived chemicals to control pest insects stems from the need for pesticide products with less negative environmental and health impacts than those of most of the highly effective synthetic insecticides. Some insecticides of plant origin were used on a large scale before they were outcompeted by synthetic insecticides. Nicotine (**41**), rotenone (**52**) and pyrethrins (**49**) have been used extensively and are effective insecticides that, because they degrade rapidly, do not accumulate in the food chain. Caution is required, however. Although many natural insecticides show lower mammalian toxicity than, for example, most organochlorine compounds, they are not harmless merely because they are natural products, a view that convincing statistical analysis has shown to be a serious misconception.[4] Another reason to remain cautious when searching for new insecticides, whether natural or not, is the risk that target insect species may become resistant to them and, still more importantly, that non-target invertebrates, including natural enemies, are at risk. Compounds that modify the behaviour of target species and have a primarily non-toxic mode of action may in the long term provide the most dependable and environmentally safe method of chemical control. Behaviourally active phytochemicals include attractants, repellents, and deterrents, several hundreds of which have been discussed in the literature.[79] We will discuss the use of feeding deterrents only as a behavioural method of insect pest management.

13.4.1 Antifeedants

Feeding deterrents or 'antifeedants' are chemicals that, when perceived, reduce or prevent insect feeding. When produced by the plant, such compounds decrease feeding damage and the risk of being infected with plant pathogens. The insect responds to the sensory detection of antifeedants by reducing food intake, which may lead either to it leaving the plant or to adverse effects on growth, development, survival, and reproduction. In contrast to repellents, antifeedants do not cause oriented locomotion away from the stimulus source.[30] In the presence of an antifeeding compound the insect may starve to death, and females may be deterred from egg-laying until they find an untreated host. Some antifeedants have been found to be effective at very low doses, in the order of less than 1.0 part per million (ppm). Azadirachtin, one of the strongest antifeedants known, inhibits feeding at 0.01 ppm in the polyphagous desert locust *Schistocerca gregaria*, when applied to palatable foliage,[78] and 1 mg of this compound suffices to protect 100 m^2 of leaf surface from this notoriously devastating insect.

Candidate compounds for an antifeedant approach to insect control must possess several essential properties (Table 13.4), which, however, are fulfilled by few if any of the compounds assayed so far.[66] As less than 1% of all secondary plant substances (estimated to number 400 000 or more) have been tested, and only on a limited number of insect species, several effective compounds may remain to be discovered. Promising chemicals that have attracted attention as potential antifeedants, whether as source material for novel analogues or not, are listed in Table 13.5.

To date only azadirachtin-based products have been marketed. Among the drimanes, polygodial (**47**), warburganal (**74**), and muzigadial (**38**) are of

Table 13.4 Criteria for antifeedant compounds as crop protectants

1. No or very low toxicity to vertebrates
2. No or very low phytotoxicity
3. Active at very low concentrations
4. Effective to many pest insect species
5. Harmless to beneficial arthropods (natural enemies, pollinators)
6. Penetration of plant surface and/or uptake by roots and systemic translocation
7. Compatible with other pest management methods
8. Limited persistence in environment
9. Sufficient source material
10. Amenable to commercial development (production costs, etc.)
11. Long shelf-life

interest. Polygodial, a sesquiterpenoid extracted from the herb water pepper (*Polygonum hydropiper*), prevents probing behaviour in aphids at very low application rate. It has been found in field trials to reduce barley yellow dwarf virus transmission by the bird-cherry aphid *Rhopalosiphum padi*, giving a 36% higher grain yield relative to untreated plots. Polygodial can be synthesized, but its action is dependent on its stereochemistry. The (+) isomer must be removed from racemic mixtures because it is, in contrast to the natural (−) isomer, highly phytotoxic.[94]

13.4.2 Neem tree, azadirachtin

Indian farmers, homemakers, and folk healers have known for centuries that neem trees have many remarkable properties, including a strong repellency to many insects. More than half a century ago an Algerian agronomist noticed that only neem trees remained unconsumed by a locust plague and showed that leaf extracts were highly unpalatable to desert locusts.[132] With the advent of DDT and a subsequent array of broad-spectrum synthetic insecticides, neem remained unnoticed as a potential source of chemicals to manipulate insects until, in the 1970s, a German entomologist, H. Schmutterer, stimulated researchers from all over the world to launch studies on the useful properties of neem.[107]

Table 13.5 Plant-derived antifeedants with promising properties for application in pest management systems, based on results of field experiments

Chemical class	Botanical source	Insects affected	Reference
Meliacins	Meliaceae	Many species	107
Drimanes	*Polygonum hydropiper*	Aphids	94
Limonoids	*Citrus paradisi*	Colorado potato beetle	80

The neem tree, *Azadirachta indica* (Meliaceae; mahogany family), probably native to Burma, has been widely cultivated for a long time in tropical Asia and Africa, where it has become extensively naturalized. It is now also widely planted in Central America, because of its rapid growth and fine timber. The tree has proved to be very adaptable and able to withstand arid conditions. Its bipinnate leaves are garlic scented when damaged, and the fruits resemble olives (Fig. 13.8).

The Meliaceae, like most sister families belonging to the order Rutales, produce and accumulate bitter and biologically active nortriterpenoids called limonoids or meliacins and quassinoids depending on structural features and occurrence. Azadirachtin, only one of more than 70 triterpenes

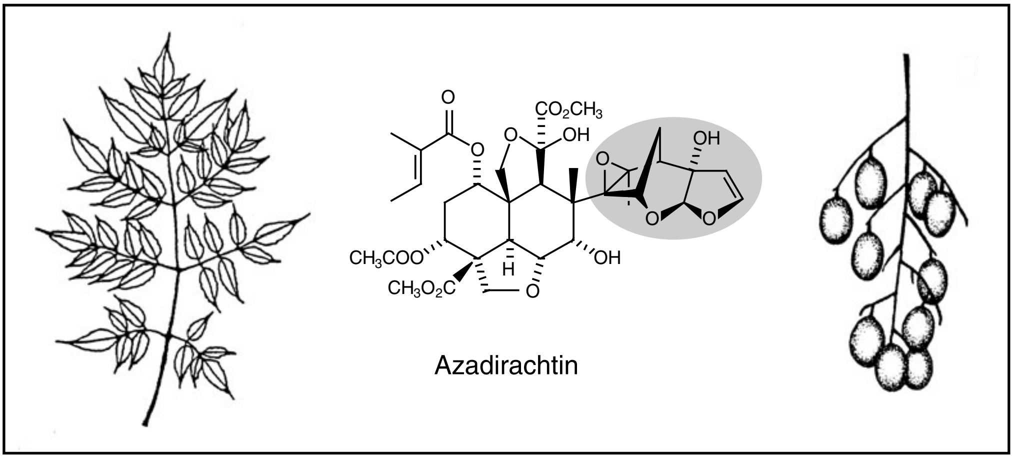


Figure 13.8 Bipinnate leaf and fruiting panicle from neem tree (*Azadirachta indica*) and structural formula of azadirachtin, a potent antifeedant and insect growth regulator. (From Schmutterer, 2002.)[107]

from neem, is a highly oxidized limonoid with many reactive functional groups in close proximity to each other (Fig. 13.8). It occurs predominantly in the seeds of *A. indica* at a concentration of about 3.5 mg per g dry kernel, and is a very potent antifeedant to many insect species, especially lepidopterous larvae and several, but not all, Orthoptera. In addition to the antifeedant action, azadirachtin and related neem-seed derivatives have often pronounced physiological effects as well. After ingestion it causes growth inhibition, malformation, disrupted reproduction, and death as a result of interference with the insect's endocrine system.[27,78,107] There is evidence suggesting that the left half of the azadirachtin molecule is the antiendocrine part, whereas the hydroxyfuranacetal moiety (the right half, i.e. the gray part of the molecule in Fig. 13.8) is particularly important for insect antifeedant activity.[17] To date, none of a large number of synthetic analogues of azadirachtin has the potency of the mother compound. The only analogue that has comparative biological activity to azadirachtin is dihydroazadirachtin. This compound is also more stable in light than azadirachtin.[111]

As a third mode of action, azadirachtin has been found to affect food utilization negatively through the inhibition of digestive enzymes.[126] To some insects, related compounds, such as salannin (**54**), which is also present in *A. indica* seeds, and toosendanin (**67**), which is isolated from the bark of the related *Melia toosendan*, are even more unpalatable than azadirachtin.[73] Whereas small farmers in the Indian continent use neem extracts in various traditional ways, there are now commercial neem products also on the market in some Western countries and several formulations have been patented.[40]

Of all plant-derived compounds known to deter insect feeding or oviposition behaviour, azadirachtin currently offers the greatest potential for widespread use.[81] In many respects it fulfils the requirements of an ideal antifeedant, notably its relative safety to beneficial organisms in the environment,[122] its practical non-toxicity to mammals at the doses applied,[19] and its systemic transport in crop plants,[6] which ensures that piercing–sucking insect species, for instance several notorious plant-hopper pests on rice, are also deterred from feeding.[106] Its sensitivity to ultraviolet light, however, necessitates the use of formulations with sun-screen filters, such as lecithin. Freshly collected seeds serve for the time being as the main source of neem compounds, but production methods employing *in vitro* tissue cultures are under way.[101] Azadirachtin has, owing to its complex molecular structure, not been synthesized chemically to date.

There is compelling evidence, as stated by Schmutterer,[107] that the neem tree 'has the potential to contribute to "solve global problems" (National Research Council, Washington, DC, 1992).' In addition, related meliaceous trees may provide similar opportunities.

13.4.3 Outlook for antifeedants as crop protectants

As discussed before (see Chapters 7 and 8), insects may, after repeated contact, habituate to the presence of a feeding deterrent. This is especially likely in polyphagous insect species[58] and would of course be a serious drawback to the usefulness of the antifeedant. Indeed, habituation to low levels of pure azadirachtin has been observed in several insect species, including the Japanese beetle *Popillia japonica*[51] and the Asian armyworm *Spodoptera litura*.[136] Interestingly, when a commercial product that contained azadirachtin as well as neem oil was tested, no habituation occurred (Fig. 13.9).[20]

In a recent study, neonate larvae of the cabbage looper *Trichoplusia ni*, another polyphagous species, were fed leaves treated with single feeding deterrents or with binary mixtures of these until the third instar, and then tested in a leaf disc choice bioassay. Larvae reared on individual antifeedants showed a significant degree of habituation, whereas those reared on binary mixtures of antifeedants did not. Clearly, such mixtures were synergistic in terms of their feeding deterrence to 'experienced' larvae.[1] This finding supports the idea that mixtures of pure allelochemicals are more effective in reducing insect feeding than pure compounds alone, a conclusion that nature discovered long ago. Natural resistance is usually mediated by a cocktail of chemicals, making

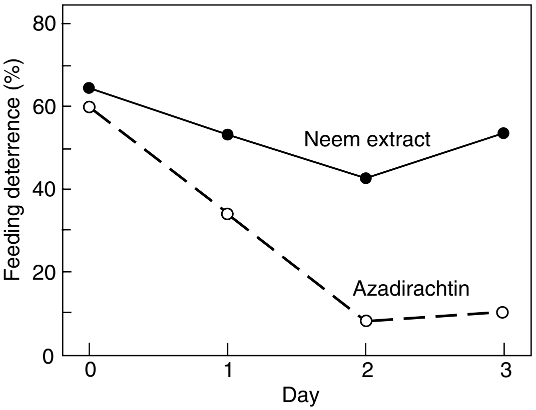


Figure 13.9 Feeding deterrence of cabbage leaf discs treated with 1.3 ng/cm^2 azadirachtin and neem seed extract containing the same absolute amount of azadirachtin in repeated-choice assays with fifth-instar larvae of *Spodoptera litura*. The values for neem do not change significantly, whereas those for azadirachtin do. (From Bomford and Isman, 1996.)[20]

adaptations by herbivores much more difficult. Therefore, behaviour-modifying substances based on two (or more) compounds are more suitable for durable crop protection than agrochemicals based on a single chemical. Neem products that contain a variety of other compounds probably offer better insect-control prospects than products based on azadirachtin alone.

Another important issue when considering the development of behaviour-modifying natural compounds for pest management is the prospect of resistance development. Long-lasting selection experiments with diamondback moth larvae (*Plutella xylostella*) showed that resistance can be developed to azadirachtin, albeit to a much lower degree than to the insecticide deltamethrin. Resistance to neem seed kernel extracts, containing a spectrum of various molecular agents, including azadirachtin, developed still more slowly than that to pure azadirachtin.[133] Presumably, the combination of behavioural and physiological actions of azadirachtin makes it more difficult for the insect to develop resistance. Unlike ordinary insecticides based on a single active ingredient, the chemical defence of plants comprises an array of compounds with varying behavioural, physiological, and toxicological properties; consequently it is more difficult for an insect to adapt to. As a matter of fact, it is unlikely that oligophagous insects, for instance, could easily develop general resistance to feeding inhibitory substances, because this would result in rapid changes of their host-plant range, which is determined primarily by the occurrence of such substances in non-host plants. However, such changes are rare events in nature.[58]

Table 13.6 Antifeedant concentration (ppm) in wheat flour wafers that reduce food intake by 50% in two locust species (data from Bernays and Chapman, 1978)[15]

	Azadirachtin	**Aristolochic acid**
Desert locust (*Schistocerca gregaria*)	0.1	0.1
Migratory locust (*Locusta migratoria*)	100	0.01

A difficulty in identifying antifeedants is that large differences exist between species in their sensitivity to a given antifeedant compound (see Tables 7.6 and 13.6).

Because most researchers, when testing candidate compounds, employ only a few or even only one insect species, effective antifeedants to a particular insect will easily escape attention. Among seven orthopterans tested for sensitivity to azadirachtin, interspecific differences span six orders of magnitude.[78] Several more caveats regarding searches for natural compounds with antifeedant activity are listed in some papers on the basics of antifeedant methodology.[40,58,66]

Is there a realistic future for any large-scale use of antifeedants? They certainly do not constitute the final tool for control of insect pests. However, in view of the environmental strains imposed by present agricultural practices, we cannot afford to leave thousands of natural defence substances provided by nature unexplored. The fact that many plant species rely to a large extent on the presence of such compounds is a strong impetus for continual explorations of the plant kingdom. Advances made on the application of neem products seem to support the statement by Frazier and Chyb[40] (p. 364) that 'The practical use of natural product feeding inhibitors in insect control is rapidly becoming a reality.' Their expectation is being fulfilled by several companies that are introducing neem products on to the pesticide market in many countries. For instance, a recent inventory lists more than 100 commercial products and their manufacturers for India alone.[91]

13.5 Weed control by herbivorous insects

Many plant species have been either purposely or accidentally transferred by humans to other parts of the world. The alien plants, once outside their natural habitat, have sometimes developed into aggressive invaders, outcompeting native plant species and causing detrimental effects in natural ecosystems or inflicting significant losses to agricultural production. In several parts of the world 60–97% of the weeds are immigrant species (Table 13.7), demonstrating that plants can become undesirable weeds in foreign habitats. As a result, crop losses to weeds exceed those attributed to insects, and expenditure on herbicides worldwide is about 30% higher than on insecticides.[97] For obvious reasons, biological control has several advantages over other types of weed control[134] and offers one of the main solutions—often the only one—to the threat of alien plant invasions.

Exotic plant species that have become weeds can sometimes be controlled by introducing host-specific insects from the plant's place of origin. Two outstandingly successful cases of control of invasive weeds by introduction of their herbivores exemplify the principle of biological weed control.

13.5.1 *Opuntia* and *Salvinia*

Prickly pears are cactus species native to North and South America. Among the 30 or so species that were introduced to Australia as pot or garden plants, two species, *Opuntia stricta* and *O. inermis*, ran out of control. *O. stricta* was brought to Australia in 1839 in a pot from the southern USA and was planted as a hedge plant in eastern Australia. It gradually developed into a pestilential weed that was difficult to control by mechanical methods, burning, etc. By 1900 it had occupied 4 million hectares in the coastal regions of Australia, and was by then spreading rapidly inland into immense areas of wheat, rangeland, and marginal agricultural land, choking out most other plant life. In 1925 some 25 million hectares were infested in Queensland and New South Wales alone. About one-half of this area was covered with dense growth, over 1 m in height and so dense as to be virtually impenetrable to humans and livestock. Farms were abandoned. In 1920 the Commonwealth Prickly Pear Board was appointed to attempt control of the weed by establishing insects and mites that feed on these cacti. Some species collected from the rich fauna present on American cacti were of some service, but a major breakthrough did not occur until the release between 1927 and 1930 of masses of a small Argentinian moth, *Cactoblastis cactorum*, whose larvae mine in the paddle-like cactus stems. Within 2 years the original stands of prickly pears had collapsed under the onslaught of the moth larvae. Successful accomplishment of the great biological control programme became apparent in 1939, and the Board was disbanded. 'Great tracts of country, utterly useless on account of the dense growth of the weed, have been brought into production. The prickly pear territory has been transformed as

Table 13.7 Origin of weed species in North America and Australia (data from Gassmann, 1995, and Pimentel, 1986)[43,96]

	No. of weeds	Origin of weeds (%)				
		Native	Europe*	America	Asia	Africa
Canada	516	40	52	4	3	0
Canada, common weeds	126	2	71	25	2	0
Australia	637	7	39	26	7	18
Australia, state of Victoria	83	4	60	23	4	10
USA, weeds in cultivated crops	80	28	50	8	5	1

* Includes species from Eurasia.

though by magic from a wilderness to a scene of prosperous endeavour' as victoriously described by Dodd.[32] At present the moth still maintains prickly pears as a scattered plant at a low, stable, equilibrium.

Since 1939 salvinia (*Salvinia molesta*), a floating aquatic fern about 2–10 cm long, native to south-eastern Brazil, has spread by human agency to many tropical and subtropical parts of the world. Outside its native range its unlimited growth has caused serious problems because it forms mats up to 1 m thick, covering whole lakes and rice paddies, and completely blocking all waterways, including slow-moving rivers and irrigation canals. Its growth capacity is evinced, for example, by its proliferation after invading the Sepik River floodplain of Papua New Guinea. A few plants introduced in 1972 grew in 8 years into mats covering 250 km^2 and weighing 2 million tonnes, severely disrupting the normal life of the local human population, which was forced to migrate and to abandon whole villages.[104] In Brazil salvinia was found to be attacked by a tiny (2 mm long) weevil species, *Cyrtobagous salviniae*, unknown before then. This insect was distributed throughout Australian salvinia infestations during the early 1980s and turned out to be an extremely effective weed-control agent. The weevil population increased in less than 1 year from a few thousand to more than 100 million individuals and destroyed 30 000 tonnes of salvinia. In Africa and India the insect also reduced the sizes of salvinia populations by more than 99%, before new, low-density, equilibria were attained.[104]

13.5.2 Success rate of biological weed-control programmes

Not all attempts at weed control by insects have met with the same spectacular successes as those described above. Even the successful establishment of an imported agent is no guarantee that it makes any impact on the abundance of the weed. Thus, although at least 69% of released arthropod species were established on alien weed plants (Fig. 13.10A), complete control was achieved on fewer occasions (Fig. 13.10B)[43,110] and the degree of control varied under different circumstances.

Although control by herbivorous insects is usually considered for introduced weed species, under certain circumstances this is also a potential method for controlling native weeds. Thus, a biocontrol programme is being developed to combat bracken (*Pteridium aquilinum*) in the UK, where this weed is becoming increasingly invasive. The very success of this plant species worldwide has resulted in its attack by different assemblages of herbivores in different parts of the world. A mesophyll-feeding

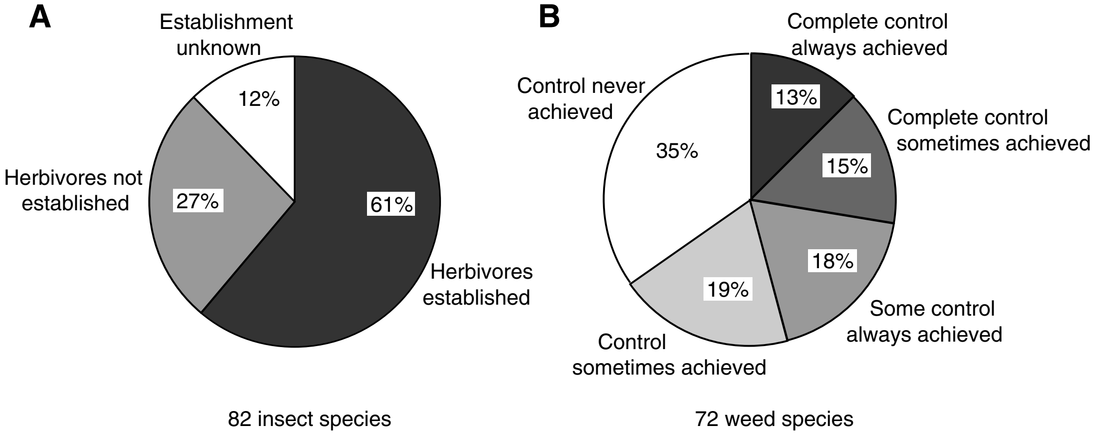


Figure 13.10 Success rates of establishment and control of invasive weeds by introduced alien herbivores. (**A**) Proportions of arthropod species established on weeds of European origin. (**B**) Degree of weed control by insects that have been introduced and established long enough to permit control assessment. (Data from Julien, 1992.)[60a]

leaf-hopper (*Eupteryx maigudoi*), native to South Africa, is one of the insect species being investigated for its suitability as a biological control agent in the UK.[38]

The advantages of weed control by employment of insect herbivores hardly need to be emphasized (Table 13.8). Its weakest point is the unpredictability of its results. This is caused by the fact that some essential demographic parameters, especially those of the functional and numerical responses, can be determined only after the herbivore has been released, because their values depend so critically on local conditions.

Some cases of negative indirect and non-target effects of weed-control programmes employing insects have raised concern about the reliability of this method. An evaluation of its value has been hindered seriously by the lack of post-release monitoring. To reduce ecological risk and reinforce the public trust in this powerful method, the need for more extensive risk and benefit assessments has recently been recognized.[26]

An interesting debate concerns the fundamental question of whether or not herbivores that are highly adapted to their hosts are likely to be the most effective control agents. Whereas highly specialized insects may flourish once confronted with unlimited food resources, it has also been argued that the plant partner in a less close insect–plant association may be more susceptible to insect attack than plant species with long-standing intimate relationships with their specialist herbivores.[38,97] *Cactoblastis cactorum*, which turned out to be very successful in controlling *Opuntia stricta* and *O. inermis*, was obtained in South America, not from either species but from a different species of *Opuntia*. However, the weevil that suppressed the invasions of salvinia is a highly adapted species, able to increase its population rapidly at its host's expense. The use of insect species that are strict monophages reduces the risk of them switching to other hosts. A complicating factor is the role of natural enemies in regulating the herbivore's population density in its native region. It is generally thought that uncoupling specialist herbivores from their normal natural enemies is a key part of biological weed control.[38]

Table 13.8 Advantages and disadvantages of biological control of weeds

Advantages
Reasonably permanent management of target species
No harmful side-effects, environmentally safe
Attack restricted to specific target species (or very small group of closely related species)
Agents are self-perpetuating, often density dependent, and self-disseminating
High benefit–cost ratios for successful programmes
Costs are non-recurrent
Disadvantages
Relatively slow-acting
If target weed is related to a crop, the number of usable herbivores is greatly reduced

Unfortunately, weed-control programmes still lack a firm theoretical basis. Perhaps an approach based on only one control agent is utterly wrong and a more diversified control system, including plant pathogens, would be more appropriate. *Lantana camara*, which developed into a pestilential weed in Hawaii, may serve as an example. Some control of this species could be obtained only after several insect species had been introduced, that together eventually constituted a large guild of herbivores. A better understanding of the factors regulating plant populations would help to improve weed-control methods by natural agents, including insects.[28]

13.6 Conclusion: diversification holds the clue to the control of pestiferous insects

As Lugenbill[72] has succinctly put it: 'Resistance to pests exists throughout nature. It is all around us in animals and plants. It needs only to be discovered and put to work to solve many of our most serious pest problems.' This chapter has indicated some approaches that elaborate on Lugenbill's perception to minimize losses to insect attack by using tools provided by nature.

The common principle for successful insect control based on biological principles is diversification.[97] Resistance breeding depends on the availability of large gene pools, and pest resistance is more effective when a variety of resistance factors are combined. Diversification of crop cultivation practices, such as intercropping and crop rotation, often reduces the risks of serious insect damage. Antifeedant compounds are more effective when applied in mixtures and when they affect various behavioural and physiological mechanisms. The limited numbers of successful examples of biological weed control possibly result from insufficient attention to the need to diversify.

This chapter has focused on the application of our knowledge of insect–plant relationships to crop production. It should be stressed that this knowledge is just as important for the management of natural ecosystems (i.e. nature conservation). Our present concern about declining biodiversity in all parts of the world requires that scientists, together with policy-makers, save what remains by protecting natural habitats, as has been convincingly and eloquently formulated by E.O. Wilson in his book *The Diversity of Life*.[138]

The key factor in sustainable agriculture—diversification—is an equally important concept underlying the management of natural ecosystems.

13.7 References

1. Akhtar, Y. and Isman, M.B. (2003). Binary mixtures of feeding deterrents mitigate the decrease in feeding deterrent response to antifeedants following prolonged exposure in the cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae). *Chemoecology*, **13**, 177–82.
2. Alonso-Blanco, C. and Koornneef, M. (2000). Naturally occurring variation in *Arabidopsis*: an underexploited resource for plant genetics. *Trends in Plant Science*, **5**, 22–9.
3. Altieri, M.A. and Nicholls, C.I. (2004). *Biodiversity and pest management in agroecosystems* (2nd edn). Food Products Press, New York.
4. Ames, B.N., Profet, M., and Gold, L.S. (1990). Dietary pesticides (99.99% all natural). *Proceedings of the National Academy of Sciences of the USA*, **87**, 7777–81.
5. Andow, D.A. (1991). Vegetational diversity and arthropod population response. *Annual Review of Entomology*, **36**, 561–86.
6. Arpaia, S. and van Loon, J.J.A. (1993). Effects of azadirachtin after systemic uptake into *Brassica oleracea* on larvae of *Pieris brassicae*. *Entomologia Experimentalis et Applicata*, **66**, 39–45.
7. Åsman, K. (2002). Trap cropping effect on oviposition behaviour of the leek moth *Acrolepiopsis assectella* and the diamondback moth *Plutella xylostella*. *Entomologia Experimentalis et Applicata*, **105**, 153–64.
8. Babu, R.M., Sajeena, A., Seetharaman, K., and Reddy, M.S. (2003). Advances in genetically engineered (transgenic) plants in pest management—an overview. *Crop Protection*, **22**, 1071–86.
9. Bach, C.E. (1980). Effects of plant density and diversity on the population dynamics of a specialist herbivore, the striped cucumber beetle, *Acalymma vittata* (Fab.). *Ecology*, **61**, 1515–30.
10. Bach, C.E. (1981). Host plant growth form and diversity: effects on abundance and feeding preference of a specialist herbivore, *Acalymma vittata* (Coleoptera: Chrysomelidae). *Oecologia*, **50**, 370–5.
11. Baliddawa, C.W. (1985). Plant species diversity and crop pest control. An analytical review. *Insect Science and its Application*, **6**, 479–87.
12. Barbosa, P. (1993). Lepidopteran foraging on plants in agroecosystems: constraints and consequences. In *Caterpillars. Ecological and evolutionary constraints on foraging* (ed. N.E. Stamp and T.M. Casey), pp. 523–66. Chapman & Hall, New York.
13. Bergelson, J. (1994). The effects of genotype and the environment on costs of resistance in lettuce. *American Naturalist*, **143**, 349–59.
14. Bernays, E.A. (1999). When host plant choice is a problem for a generalist herbivore: experiments with the whitefly, *Bemisia tabaci*. *Ecological Entomology*, **24**, 260–7.
15. Bernays, E.A. and Chapman, R.F. (1978). Plant chemistry and acridoid feeding behaviour. In *Biochemical aspects of plant–animal coevolution* (ed. J. Harborne), pp. 99–141. Academic Press, New York.
16. Bird, J.V. (1982). Multi-adversity (diseases, insects and stresses) resistance (MAR) in cotton. *Plant Diseases*, **66**, 173–6.
17. Blaney, W.M., Simmonds, M.S.J., Ley, S.V., Anderson, J.C., Smith, S.C., and Wood, A. (1994). Effect of azadirachtin-derived decalin (perhydronaphthalene) and dihydrofuranacetal (furo[2,3-b] pyran) fragments on the feeding behaviour of *Spodoptera littoralis*. *Pesticide Science*, **40**, 169–73.
18. Boeke, S.J., Van Loon, J.J.A., Van Huis, A., Kosu, D.K., and Dicke, M. (2001). Review of the use of plant material to protect stored seeds, with a focus on *Callosobruchus maculatus* infesting cowpea. *Wageningen University Papers*, **2001–3**, 1–108.
19. Boeke, S.J., Boersma, M.G., Alink, G.M., Van Loon, J.J.A., Van Huis, A., Dicke, M., *et al.* (2004). Safety evaluation of neem (*Azadirachta indica*) derived pesticides. *Journal of Ethnopharmacology*, **94**, 25–41.

20. Bomford, M.K. and Isman, M.B. (1996). Desensitization of fifth instar *Spodoptera litura* (Lepidoptera: Noctuidae) to azadirachtin and neem. *Entomologia Experimentalis et Applicata*, **81**, 307–13.
21. Bourguet, D. (2004). Resistance to *Bacillus thuringiensis* toxins in the European corn borer: what chance for *Bt* maize? *Physiological Entomology*, **29**, 251–6.
22. Carroll, S.P. and Dingle, H. (1996). The biology of post-invasion events. *Biological Conservation*, **78**, 207–14.
23. Chew, F. (1995). From weeds to crops: changing habitats of pierid butterflies (Lepidoptera: Pieridae). *Journal of the Lepidopterists' Society*, **49**, 285–303.
24. Coll, M. (1998). Parasitoid activity and plant species composition in intercropped systems. In *Enhancing biological control: habitat management to promote natural enemies of agricultural pests* (ed. C.H. Pickett and R.L. Bugg), pp. 85–119. University of California Press, Berkeley, CA.
25. Coll, M. and Bottrell, D.G. (1996). Movement of an insect parasitoid in simple and diverse plant assemblages. *Ecological Entomology*, **21**, 141–9.
26. Cory, J.S. and Myers, J.H. (2000). Direct and indirect ecological effects of biological control. *Trends in Ecology and Evolution*, **15**, 137–9.
27. Cowles, R.S. (2004). Impact of azadirachtin on vine weevil (Coleoptera: Curculionidae) reproduction. *Agricultural and Forest Entomology*, **6**, 291–4.
28. Crawley, M.J. (1983). *Herbivory. The dynamics of animal–plant interactions*. Blackwell Scientific, Oxford.
29. Den Nijs, H.C.M., Bartsch, D., and Sweet, J. (2004). *Introgression from genetically modified plants into wild relatives*. CAB International, Wallingford.
30. Dethier, V.G., Barton Browne, L., and Smith, C.N. (1960). The designation of chemicals in terms of the responses they elicit from insects. *Journa of Economic Entomology*, **53**, 134–6.
31. Dicke, M. (1999). Direct and indirect effects of plants on performance of beneficial organisms. In *Handbook of pest management* (ed. J.R. Ruberson), pp. 105–53. Marcel Dekker, New York.
32. Dodd, A.P. (1940). *The biological campaign against prickly pear*. Government Printer, Brisbane.
33. Dover, J.W. (1986). The effect of labiate herbs and white clover on *Plutella xylostella* oviposition. *Entomologia Experimentalis et Applicata*, **42**, 243–7.
34. Dunn, J.A. and Kempton, D.P.H. (1972). Resistance to attack by *Brevicoryne brassicae* among plants of Brussels sprouts. *Annals of Applied Biology*, **72**, 1–11.
35. Eigenbrode, S.D. (2002). Resistance management in host–plant resistance. In *Encyclopedia of pest management* (ed. D. Pimentel), pp. 708–11. Dekker, New York.
36. Eigenbrode, S.D. and Clement, S.L. (1999). Plant genetic resources for the study of insect–plant interactions. In *Global plant genetic resources for insect-resistant crops* (ed. S.L. Clement and S.S. Quisenberry), pp. 243–62. CRC Press, Boca Raton, FL.
37. Ekbom, B., Irwin, M.E., and Robert, Y. (2000). *Interchanges of insects between agricultural and surrounding landscapes*. Kluwer Academic, Dordrecht.
38. Fowler, S.V. (1993). The potential for control of bracken in the UK using introduced herbivorous insects. *Pesticide Science*, **37**, 393–7.
39. França, F.H., Plaisted, R.L., Roush, R.T., Via, S., and Tingey, W.M. (1994). Selection response of the Colorado potato beetle for adaptation to the resistant potato, *Solanum berthaultii. Entomologia Experimentalis et Applicata*, **73**, 101–9.
40. Frazier, J.L. and Chyb, S. (1995). Use of feeding inhibitors in insect control. In *Regulatory mechanisms in insect feeding* (ed. R.F. Chapman and G. de Boer), pp. 364–81. Chapman & Hall, London.
41. Fritzsche, R., Decker, H., Lehmann, W., Karl, E., and Geissler, K. (1988). *Resistenz von Kulturpflanzen gegen Tierische Schaderreger*. Springer, Berlin.
42. Garcia, M.A. and Altieri, M.A. (1992). Explaining differences in flea beetle *Phyllotreta cruciferae* Goeze densities in simple and mixed broccoli cropping systems as a function of individual behavior. *Entomologia Experimentalis et Applicata*, **62**, 201–9.
43. Gassmann, A. (1995). Europe as a source of biological control agents of exotic invasive weeds: status and implications. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft*, **68**, 313–22.
44. Glinwood, R., Ninkovic, V., Pettersson, J., and Ahmed, A. (2004). Barley exposed to aerial allelopathy from thistles (*Cirsium* spp.) becomes less acceptable to aphids. *Ecological Entomology*, **29**, 188–95.
45. Gold, C.S., Altieri, M.A., and Bellotti, A.C. (1989). Effects of cassava varietal mixtures on the whiteflies *Aleurotrachelus socialis* and *Trialeurodes variabilis* in Colombia. *Entomologia Experimentalis et Applicata*, **53**, 195–202.
46. Gould, F. (1986). Simulation models for predicting durability of insect-resistant germ plasm: a deterministic diploid, two-locus model. *Environmental Entomology*, **15**, 1–10.
47. Gould, F. (1988). Genetics of pairwise and multispecies plant–herbivore coevolution. In *Chemical mediation of coevolution* (ed. K.C. Spencer), pp. 13–55. Academic Press, San Diego, CA.

48. Groot, A.T. and Dicke, M. (2002). Insect-resistant transgenic plants in a multitrophic context. *Plant Journal*, **31**, 387–406.
49. Haq, S.K., Atif, S.M., and Khan, R.H. (2004). Protein proteinase inhibitor genes in combat against insects, pests, and pathogens: natural and engineered phytoprotection. *Archives of Biochemistry and Biophysics*, **431**, 145–59.
50. Hastings, A. (1999). Outbreaks of insects: a dynamic approach. In *Theoretical approaches to biological control* (ed. B.A. Hawkins and H.V. Cornell), pp. 206–15. Cambridge University Press, Cambridge.
51. Held, D.W., Eaton, T., and Potter, D.A. (2001). Potential for habituation to a neem-based feeding deterrent in Japanese beetles, *Popillia japonica*. *Entomologia Experimentalis et Applicata*, **101**, 25–32.
52. Hill, D.E. (1997). *The economic importance of insects*. Chapman & Hall, London.
53. Hokkanen, H.M.T. (1991). Trap cropping in pest management. *Annual Review of Entomology*, **36**, 119–38.
54. Hooks, C.R.R. and Johnson, M.W. (2003). Impact of agricultural diversification on the insect community of cruciferous crops. *Crop Protection*, **22**, 223–38.
55. Howe, W.L., Sanborn, J.R., and Rhodes, A.M. (1976). Western corn rootworm adult and spotted cucumber beetle associations with *Cucurbita* and cucurbitacins. *Environmental Entomology*, **5**, 1043–8.
56. James, C. (2004). *Preview: Global status of commercialized biotech/GM crops: 2003*. ISAAA Briefs No. 32. Ithaca, NY.
57. Jena, K.K. and Khush, G.S. (1990). Introgression of genes from *Oryza officinalis* Well × Watt to cultivated rice, *O. sativa* L. *Theoretical and Applied Genetics*, **80**, 737–45.
58. Jermy, T. (1990). Prospects of antifeedant approach to pest control—a critical review. *Journal of Chemical Ecology*, **16**, 3151–66.
59. Johns, T. and Alonso, J.G. (1990). Glycoalkaloid change during domestication of the potato, *Solanum* section Petota. *Euphytica*, **50S**, 203–10.
60. Josefsson, E. (1967). Distribution of thioglucosides in different parts of *Brassica* plants. *Phytochemistry*, **6**, 1617–27.

60a. Julien, M.H. (1992). *Biological control of weeds* (3rd edn). CAB International, Wallingford.

61. Julien, M.H. and Griffiths, M.W. (1998). *Biological control of weeds: a world catalogue of agents and their target weeds* (4th edn). CAB International, Wallingford.
62. Kim, K.C. and McPheron, B.A. (1993). *Evolution of insect pests. Patterns of variation*. John Wiley, New York.
63. Kogan, M. (1986). Plant defense strategies and host-plant resistance. In *Ecological theory and integrated pest management practice* (ed. M. Kogan), pp. 83–134. John Wiley, New York.
64. Kogan, M. and Ortman, E.F. (1978). Antixenosis—a new term proposed to define Painter's 'non-preference' modality of resistance. *Bulletin of the Entomological Society of America*, **24**, 175–6.
65. Kota, M., Daniell, H., Varma, S., Garczynski, S.F., Gould, F., and Moar, W.J. (1999). Overexpression of the *Bacillus thuringiensis (Bt)* Cry2Aa2 protein in chloroplasts confers resistance to plants against susceptible and *Bt*-resistance insects. *Proceedings of the National Academy of Sciences of the USA*, **96**, 1840–5.
66. Koul, O. (2004). *Insect antifeedants*. CRC Press, Boca Raton, FL.
67. Kowalchuk, G.A., Bruinsma, M., and Van Veen, J.A. (2003). Assessing responses of soil microorganisms to GM plants. *Trends in Ecology and Evolution*, **18**, 403–10.
68. Koziel, M.G., Beland, G.L., Bowman, C., Carozzi, N.B., Crenshaw, R., Crossland, L., *et al*. (1993). Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Bio/Technology*, **11**, 194–200.
69. Krips, O.E., Willems, P.E.L., Gols R., Posthumus, M.A., Gort, G., and Dicke, M. (2001). Comparison of cultivars of ornamental crop *Gerbera jamesonii* on production of spider-mite induced volatiles, and their attractiveness to the predator *Phytoseiulus persimilis*. *Journal of Chemical Ecology*, **27**, 1355–72.
70. Liu, X.M., Smith, C.M., Gill, B.S., and Tolmay, V. (2001). Microsatellite markers linked to six Russian wheat aphid resistance genes in wheat. *Theoretical and Applied Genetics*, **102**, 504–10.
71. Lovett, J.V. and Hoult, A.H.C. (1992). Gramine: the occurrence of a self defence chemical in barley, *Hordeum vulgare* L. *Proceedings of the Sixth Australian Society of Agrononomists Conferences*, Armidale, pp. 426–9.
72. Lugenbill, P. (1969). Developing resistant plants—the ideal method for controlling insects. *USDA, Agricultural Research Service and Product Research Report*, **111**, 1–14.
73. Luo, L.E., van Loon, J.J.A., and Schoonhoven, L.M. (1995). Behavioural and sensory responses to some neem compounds by *Pieris brassicae* larvae. *Physiological Entomology*, **20**, 134–40.
74. Mauricio, R. and Rausher, M.D. (1997). Experimental manipulation of putative selective agents provide evidence for the role of natural enemies in the evolution of plant defense. *Evolution*, **51**, 1435–44.
75. Maxwell, F.G. and Jennings, P.R. (1980). *Breeding plants resistant to insects*. John Wiley, New York.

76. Metz, M. (2003). Bacillus thuringiensis: *the cornerstone of modern agriculture*. Haworth Press, Binghampton, NY.
77. Morandini, P. and Salamini, F. (2003). Plant biotechnology and breeding: allied for years to come. *Trends in Plant Science*, **8**, 70–5.
78. Mordue (Luntz), A.J. and Blackwell, A. (1993). Azadirachtin: an update. *Journal of Insect Physiology*, **39**, 903–24.
79. Morgan, E.A. and Mandava, N.B. (1990). *CRC handbook of natural pesticides*, Vol. 4, *Insect attractants and repellents*. CRC Press, Boca Raton, FL.
80. Murray, K.D., Alford, A.R., Groden, E., Drummond, F.A., Storch, R.H., Bentley, M.D., *et al.* (1993). Interactive effects of an antifeedant used with *Bacillus thuringiensis* var. *san diego* delta endotoxin on Colorado potato beetle (Coleoptera: Chrysomelidae). *Journal of Economic Entomology*, **86**, 1793–801.
81. National Research Council (1992). *Neem: a tree for solving problems*. National Academy Press, Washington, DC.
82. Needham, J. (1986). *Science and civilization in China*, Vol. 6(1). Cambridge University Press, Cambridge.
83. Nicholls, C.I., Parrella, M.P., and Altieri, M.A. (2000). Reducing the abundance of leafhoppers and thrips in a northern California organic vineyard through maintenance of full season floral diversity with summer cover crops. *Agricultural and Forest Entomology*, **2**, 107–13.
84. Ninkovic, V., Olsson, U., and Pettersson, J. (2002). Mixing barley cultivars affects aphid host plant acceptance in field experiments. *Entomologia Experimentalis et Applicata*, **102**, 177–82.
85. O'Brien, D.M., Boggs, C.L., and Fogel, M.L. (2004). Making eggs from nectar: the role of life history and dietary carbon turnover in butterfly reproductive resource allocation. *Oikos*, **105**, 279–91.
86. O'Callaghan, M., Glare, T.R., Burgess, E.P.J., and Malone, L.A. (2005). Effects of plants genetically modified for insect resistance on nontarget organisms. *Annual Review of Entomology*, **50**, 271–92.
87. Oerke, E.C. and Dehne, H.W. (1997). Global crop production and the efficacy of crop protection—current situation and future trends. *European Journal of Plant Pathology*, **103**, 203–15.
88. Oerke, E.C., Dehne, H.W., Schönbeck, F., and Weber, A. (1994). *Crop production and crop protection. Estimated losses in major food and cash crops*. Elsevier, Amsterdam.
89. Painter, R.H. (1951). *Insect resistance in crop plants*. University Press of Kansas, Lawrence.
90. Panda, N. and Khush, G.S. (1995). *Host plant resistance to insects*. CAB International, Wallingford.
91. Parmar, B.S. and Sinha, S. (2002). Results with commercial neem formulations produced in India. In *The neem tree*, Azadirachta indica *A. Juss. and other meliaceous plants* (2nd edn) (ed. H. Schmutterer), pp. 562–92. Neem Foundation, Mumbai.
92. Perlak, F.J., Deaton, R.W., Armstrong, T.A., Fuchs, R.L., Sims, S.R., Greenplate, J.T., *et al.* (1990). Insect resistant cotton plants. *Bio/Technology*, **8**, 939–43.
93. Perlak, F.J., Fuchs, R.L., Dean, D.A., McPherson, S.L., and Fischhoff, D.A. (1991). Modification of the coding sequence enhances plant expression of insect control protein genes. *Proceedings of the National Academy of Sciences of the USA*, **88**, 3324–8.
94. Pickett, J.A., Dawson, G.W., Griffiths, D.C., Hassanali, A., Merritt, L.A., Mudd, A., *et al.* (1987). Development of plant antifeedants for crop protection. In *Pesticide science and biotechnology* (ed. R. Greenhalgh and T.R. Roberts), pp. 125–8. Blackwell, Oxford.
95. Pilson, D. and Prendeville, H.R. (2004). Ecological effects of transgenic crops and the escape of transgenes into wild populations. *Annual Review of Ecology, Evolution and Systematics*, **35**, 149–74.
96. Pimentel, D. (1986). Biological invasions of plants and animals in agriculture and forestry. In *Ecology of biological invasions of North America and Hawaii* (ed. H.A. Mooney and J.A. Drake), pp. 149–62. Springer, New York.
97. Pimentel, D. (1991). Diversification of biological control strategies in agriculture. *Crop Protection*, **10**, 243–53.
98. Pimentel, D. (1993). Habitat factors in new pest invasions. In *Evolution of insect pests. Patterns of variation* (ed. K.C. Kim and B.A. McPheron), pp. 165–81. John Wiley, New York.
99. Pimentel, D. (ed.) (1997). *Techniques for reducing pesticides: environmental and economic benefits*. John Wiley, Chichester.
100. Quiros, C.F., Stevens, M.A., Rick, C.M., and Kok-Yokomi, M.K. (1977). Resistance in tomato to the pink form of the potato aphid (*Macrosiphum euphorbiae* Thomas): the role of anatomy, epidermal hairs, and foliage composition. *Journal of the American Society of Horticultural Science*, **102**, 166–71.
101. Raval, K.N., Hellwig, S., Prakash, G., Ramos-Plasencia, A., Srivastava, A., and Büchs, J. (2003). Necessity of a two-stage process for the production of azadirachtin-related limonoids in suspension cultures of *Azadirachta indica*. *Journal of Bioscience and Bioengineering*, **96**, 16–22.

102. Rea, J.H., Wratten, S.D., Sedcole, R., Cameron, P.J., Davis, S.I., and Chapman, R.B. (2002). Trap cropping to manage green vegetable bug *Nezara viridula* (L.) (Heteroptera: Pentatomidae) in sweet corn in New Zealand. *Agricultural and Forest Entomology*, **4**, 101–7.
103. Risch, S.J. (1987). Agricultural ecology and insect outbreaks. In *Insect outbreaks* (ed. P. Barbosa and J.C. Schultz), pp. 217–38. Academic Press, San Diego, CA.
104. Room, P.M. (1990). Ecology of a simple plant–herbivore system: biological control of *Salvinia. Trends in Ecology and Evolution*, **5**, 74–9.
105. Root, R. (1973). Organization of a plant–arthropod association in simple and diverse habitats. The fauna of collards. *Ecological Monographs*, **43**, 95–124.
106. Saxena, R.C. (1987). Antifeedants in tropical pest management. *Insect Science and its Application*, **8**, 731–6.
107. Schmutterer, H. (ed.) (2002). *The neem tree*, Azadirachta indica *A. Juss. and other meliaceous plants; sources of unique natural products for integrated pest management, medicine, industry and other purposes* (2nd edn). Neem Foundation, Mumbai.
108. Schroeder, H.E., Gollasch, S., Moore, A., Tabe, L.M., Craig, S., Hardie, D.C., *et al*. (1995). Bean α-amylase inhibitor confers resistance to the pea weevil (*Bruchus pisorum*) in transgenic peas (*Pisum sativum* L.). *Plant Physiology*, **107**, 1233–40.
109. Sharma, H.C., Sharma, K.K., and Crouch, J.H. (2004). Genetic transformation of crops for insect resistance: potential and limitations. *Critical Reviews in Plant Sciences*, **23**, 47–72.
110. Sheppard, A.W. (1992). Predicting biological weed control. *Trends in Ecology and Evolution*, **7**, 290–1.
111. Simmonds, M.S.J. (2000). Molecular- and chemo-systematics: do they have a role in agrochemical discovery? *Crop Protection*, **19**, 591–6.
112. Smith, A.E. and Secoy, D.M. (1981). Plants used for agricultural pest control in western Europe before 1850. *Chemistry and Industry*, **3 January 1981**, 12–17.
113. Smith, C.M. (1989). *Plant resistance to insects. A fundamental approach*. John Wiley, New York.
114. Smith, C.M. and Quisenberry, S.S. (1994). The value and use of plant resistance to insects in integrated crop management. *Journal of Agricultural Entomology*, **11**, 189–90.
115. Smith, C.M., Khan, Z.R., and Pathak, M.D. (1994). *Techniques for evaluating insect resistance in crop plants*. CRC Press, Boca Raton, FL.
116. Southwood, T.R.E. (1973). The insect/plant relationship—an evolutionary perspective. *Symposia of the Royal Entomological Society of London*, **6**, 3–30.
117. Stoner, K.A. (1992). Bibliography of plant resistance to arthropods in vegetables, 1977–1991. *Phytoparasitica*, **20**, 125–80.
118. Stowe, K.A., Marquis, R.J., Hochwender, C.G., and Simms, E.L. (2000). The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology and Systematics*, **31**, 565–95.
119. Sunderland, K. and Samu, F. (2000). Effects of agricultural diversification on the abundance, distribution, and pest control potential of spiders: a review. *Entomologia Experimentalis et Applicata*, **95**, 1–13.
120. Tabashnik, B.E., Liu, Y.-B., Finson, N., Masson, L., and Heckel, D.G. (1997). One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. *Proceedings of the National Academy of Sciences of the USA*, **94**, 1640–4.
121. Tabashnik, B.E., Carrière, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., *et al*. (2003). Insect resistance to transgenic *Bt* crops: lessons from the laboratory and field. *Journal of Economic Entomology*, **96**, 1031–8.
122. Tang, Y.Q., Weathersbee, A.A., and Mayer, R.T. (2002). Effect of neem seed extract on the brown citrus aphid (Homoptera: Aphididae) and its parasitoid *Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae). *Environmental Entomology*, **31**, 172–6.
123. Thiéry, D. and Visser, J.H. (1987). Misleading the Colorado potato beetle with an odor blend. *Journal of Chemical Ecology*, **13**, 1139–46.
124. Thies, C. and Tscharntke, T. (1999). Landscape structure and biological control in agrosystems. *Science*, **285**, 893–5.
125. Thresh, J.M. (1981). *Pests, pathogens and vegetation: the role of weeds and wild plants in the ecology of crop pests and diseases*. Pitman, Boston, MA.
126. Timmins, W.A. and Reynolds, S.E. (1992). Azadirachtin inhibits secretion of trypsin in midgut of *Manduca sexta* caterpillars: reduced growth due to impaired protein digestion. *Entomologia Experimentalis et Applicata*, **63**, 47–54.
127. Tonhasca, A. and Byrne, D.A. (1994). The effects of crop diversification on herbivorous insects: a meta-analysis approach. *Ecological Entomology*, **19**, 239–44.
128. Trujillo-Arriaga, J. and Altieri, M.A. (1990). A comparison of aphidophagous arthropods on maize polycultures and monocultures, in Central Mexico. *Agriculture Ecosystems & Environment*, **31**, 337–49.

129. Tuxill, J. (1999). *Nature's cornucopia: our stake in plant diversity*. Worldwatch Institute, Washington, DC.
130. Uvah, I.I.I. and Coaker, T.H. (1984). Effect of mixed cropping on some insect pests of carrots and onions. *Entomologia Experimentalis et Applicata*, **36**, 159–67.
131. Vandermeer, J. (1989). *The ecology of intercropping*. Cambridge University Press, Cambridge.
132. Volkonsky, M. (1937). Sur l'action acridifuge des extraits de feuilles de *Melia azedarach*. *Archives de l'Institut Pasteur d'Algérie*, **15**, 427–32.
133. Völlinger, M. and Schmutterer, H. (2002) Development of resistance to azadirachtin and other neem ingredients. In *The neem tree*, Azadirachta indica *A. Juss. and other meliaceous plants; sources of unique natural products for integrated pest management, medicine, industry and other purposes* (2nd edn) (ed. H. Schmutterer), pp. 598–606. Neem Foundation, Mumbai.
134. Wapshere, A.J., Delfosse, E.S., and Cullen, J.M. (1989). Recent developments in biological control of weeds. *Crop Protection*, **8**, 227–50.
135. Welter, S.C. (2001). Contrasting plant responses to herbivory in wild and domesticated habitats. In *Biotic stress and yield loss* (ed. R.K.D. Peterson and L.G. Higley), pp. 160–84. CRC Press, Boca Raton, FL.
136. Wheeler, D.A. and Isman, M.B. (2000). Effect of *Trichilia americana* extract on feeding behavior of Asian armyworm, *Spodoptera litura*. *Journal of Chemical Ecology*, **26**, 2791–800.
137. Williams, W.G., Kennedy, G.G., Yamamoto, R.T., Thacker, J.D., and Bordner, J. (1980). 2-Tridecanone: a naturally occurring insecticide from the wild tomato *Lycopersicon hirsutum* f. *glabratum*. *Science*, **207**, 888–9.
138. Wilson, E.O. (1992). *The diversity of life*. Penguin Books, London.
139. Windle, P.N. and Franz, E.H. (1979). The effects of insect parasitism on plant competition: greenbugs and barley. *Ecology*, **60**, 521–9.
140. Wink, M. (1988). Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. *Theoretical and Applied Genetics*, **75**, 225–33.
141. Wyss, E. (1995). The effects of weed strips on aphids and aphidophagous predators in an apple orchard. *Entomologia Experimentalis et Applicata*, **75**, 43–9.

Appendix A: Further reading

Books that focus wholly or to a large extent on insect–plant interactions

Abrahamson, W.G. (ed.) (1989). *Plant–animal interactions*. McGraw-Hill, New York, 480 pp.

Ahmad, S. (ed.) (1983). *Herbivorous insects. Host seeking behavior and mechanisms*. Academic Press, San Diego, 257 pp.

Ananthakrishnan, T.N. (1992). *Dimensions of insect–plant interactions*. Oxford and IBH Publishing, New Delhi, 184 pp.

Ananthakrishnan, T.N. (1994). *Functional dynamics of phytophagous insects*. Oxford and IBH Publishing, New Delhi, 304 pp.

Ananthakrishnan, T.N. (ed.) (2001). *Insects and plant defense dynamics*. Oxford and IBH Publishing, New Delhi, 256 pp.

Ananthakrishnan, T.N. and Raman, A. (eds) (1993). *Chemical ecology of phytophagous insects*. Science Publishers, Lebanon, NH, 332 pp.

Barbosa, P. and Letourneau, D.K. (eds) (1988) *Novel aspects of insect–plant interactions*. John Wiley, New York, 362 pp.

Barbosa, P. and Schultz, J.C. (eds) (1987). *Insect outbreaks*. Academic Press, San Diego, 578 pp.

Barbosa, P. and Wagner, M.R. (1989). *Introduction to forest and shade tree insects*. Academic Press, San Diego, 639 pp.

Barbosa, P., Krischik, V.A., and Jones, C.G. (eds) (1991). *Microbial mediation of plant–herbivore interactions*. John Wiley, New York, 530 pp.

Bernays, E.A. (ed.) (1989–1994). *Insect–plant interactions*, Vols 1–5. CRC Press, Boca Raton, 164, 199, 258, 240, 240 pp.

Bernays, E.A. and Chapman, R.F. (1994). *Host-plant selection by phytophagous insects*. Chapman & Hall, New York, 312 pp.

Boethel, D.J. and Eikenbary, R.D. (eds) (1986). *Interactions of plant resistance and parasitoids and predators of insects*. Horwood, Chichester, 224 pp.

Brattsten, L.B. and Ahmad, S. (eds) (1986). *Molecular aspects of insect–plant interactions*. Plenum Press, New York, 346 pp.

Brues, C.T. (1946). *Insect dietary*. Harvard University Press, Massachusetts (reprinted in 1972 as *Insects, food and ecology*, Dover, New York), 466 pp.

Chadwick, D.J. and Goode, J.A. (eds) (1999). *Insect–plant interactions and inducible plant defence*. John Wiley, Chichester, 281 pp.

Crawley, M.J. (1983). *Herbivory. The dynamics of animal–plant interactions*. Blackwell, Oxford, 437 pp.

Dajoz, R. (2000). *Insects and forests. The role and diversity of insects in the forest environment*. Intercept, Lavoisier, 680 pp.

Denno, R.F. and McClure, M.S. (eds) (1983). *Variable plants and herbivores in natural and managed systems*. Academic Press, New York, 717 pp.

Edwards, P.J. and Wratten, S.D. (1983). *Insect/plant relationships*. Edward Arnold, London, 60 pp.

Fritz, R.S. and Simms, E.L. (eds) (1992). *Plant resistance to herbivores and pathogens. Ecology, evolution and genetics*. University of Chicago Press, Chicago, 590 pp.

Gilbert, L.E. and Raven, P.H. (eds) (1975). *Coevolution of animals and plants*. University of Texas Press, Austin, 246 pp.

Green, M.B. and Hedin, P.A. (eds) (1986). *Natural resistance of plants to pests: role of allelochemics*. ACS Symposium 246, Washington, DC.

Harborne, J.B. (ed.) (1978). *Biochemical aspects of plant and animal coevolution*. Academic Press, New York, 435 pp.

Hedin, P.A. (ed.) (1983). *Plant resistance to insects*. ACS, Symposium 208, 375 pp.

Heinrichs, E.A. (ed.) (1988). *Plant stress–insect interaction*. John Wiley, New York, 492 pp.

Herrera, C.M. and Pellmyr, O. (eds) (2002). *Plant–animal interactions—an evolutionary approach*. Blackwell Science, Oxford, 313 pp.

Hodkinson, I.D. and Hughes, M.K. (1982). *Insect herbivory*. Chapman & Hall, London, 77 pp.

Howe, H.F. and Westley, L.C. (1988). *Ecological relationships of plants and animals*. Oxford University Press, Oxford, 273 pp.

Jolivet, P. (1992). *Insects and plants. Parallel evolution and adaptations* (2nd edn). CRC Press, Boca Raton, 208 pp.

Jolivet, P. (1999). *Interrelationship between insects and plants*. CRC Press, Boca Raton, 309 pp.

Juniper, B.E. and Southwood, T.R.E. (eds) (1986). *Insects and the plant surface*. Edward Arnold, London, 360 pp.

Karban, R. and Baldwin, I.T. (1997). *Induced responses to herbivory*. University of Chicago Press, Chicago, 319 pp.

Kim, K.C. and McPheron, B.A. (eds) (1993). *Evolution of insect pests. Patterns of variation*. John Wiley, New York, 479 pp.

Mattson, W.J., Levieux, J., and Bernard-Degan, C. (eds) (1988). *Mechanisms of woody plant defenses against insects*. Springer, Berlin, 416 pp.

Maxwell, F.G. and Jennings, P.R. (eds) (1980). *Breeding plants resistant to insects*. John Wiley, New York, 683 pp.

Metcalf, R.L. and Metcalf, E.R. (1992). *Plant kairomones in insect ecology and control*. Chapman & Hall, London, 168 pp.

Miller, J.R. and Miller, T.A. (eds) (1986). *Insect–plant interactions*. Springer, Berlin, 342 pp.

Painter, R.H. (1951). *Insect resistance in crop plants*. University Press of Kansas, Lawrence, 520 pp.

New, T.R. (n.y., ±1989). *Associations between insects and plants*. New South Wales University Press, Kensington, NSW, 113 pp.

Olff, H., Brown, V.K., and Drent, R.H. (eds) (1999). *Herbivores: between plants and predators*. Blackwell, Oxford, 639 pp.

Panda, N. and Khush, G.S. (1995). *Host plant resistance to insects*. CAB International, Wallingford, 431 pp.

Peterson, R.K.D. and Higley, L.G. (eds) (2001). *Biotic stress and yield loss*. CRC Press, Boca Raton, FL, 261 pp.

Raman, A. (ed.) (1997). *Ecology and evolution of plant-feeding insects in natural and man-made environments*. Backhuys, Leiden, 245 pp.

Rosenthal, G.A. and Berenbaum, M.R. (eds) (1991, 1992). *Herbivores. Their interactions with secondary plant metabolites* (2nd edn). Academic Press, New York, 468 & 493 pp.

Rosenthal, G.A. and Janzen, D.H. (eds) (1979). *Herbivores. Their interaction with secondary plant metabolites*. Academic Press, New York, 718 pp.

Smith, C.M. (1984). *Plant resistance to insects. A fundamental approach*. John Wiley, New York, 286 pp.

Spencer, K.V. (ed.) (1988). *Chemical mediation of coevolution*. Academic Press, New York, 609 pp.

Stamp, N.E. and Casey, T.M. (eds) (1993). *Caterpillars. Ecological and evolutionary constraints on foraging*. Chapman & Hall, New York, 587 pp.

Stork, N.E., Adis, J., and Didham, R.K. (eds) (1997). *Canopy arthropods*. Chapman & Hall, London, 567 pp.

Strong, D.R., Lawton, J.H., and Southwood, T.R.E. (1984). *Insects on plants. Community patterns and mechanisms*. Blackwell, Oxford, 313 pp.

Tallamy, D.W. and Raupp, M.J. (eds) (1991). *Phytochemical induction by herbivores*. Wiley, New York, 431 pp.

Tscharntke, T. and Hawkins, B.A. (eds) (2002). *Multitrophic level interactions*. Cambridge University Press, Cambridge, 274 pp.

Van Emden, H.F. (ed.) (1973). *Insect/plant relationships*. Symposia of the Royal Entomological Society of London, 6. Blackwell, Oxford, 213 pp.

Wallace, J.W. and Mansell, R.L. (eds) (1976). *Biochemical interaction between plants and insects*. Plenum, New York, 425 pp.

Watt, A.D., Stork, N.E., and Hunter, M.D. (eds) (1997). *Forests and insects*. Chapman & Hall, London, 406 pp.

Proceedings of international symposia on insect–plant relationships

Proceedings of the 1st International Symposium, Insect and Foodplant, Wageningen, 1957 (ed. J. de Wilde). *Entomologia Experimentalis et Applicata*, **1**, 1–118 (1958).

Proceedings of the 2nd International Symposium, Insect and Host Plant, Wageningen, 1969 (ed. J. de Wilde and L.M. Schoonhoven). *Entomologia Experimentalis et Applicata*, **12**, 471–810 (1969).

Proceedings of the 3rd International Symposium, The Host-Plant in Relation to Insect Behaviour and Reproduction, Budapest, 1974 (ed. T. Jermy). Plenum Press, New York. Also: *Symposia Biologica Hungarica*, **16**, 1–322 (1976).

Proceedings of the 4th International Symposium, Insect and Host Plant, Slough, 1978 (eds. R.F. Chapman and E.A. Bernays). *Entomologia Experimentalis et Applicata*, **24**, 201–766 (1978).

Proceedings of the 5th International Symposium, Insect–Plant Relationhips, Wageningen, 1982 (ed. J.H. Visser and A.K. Minks). Pudoc, Wageningen (1982).

Proceedings of the 6th International Symposium, Insects–Plants, Pau, 1986 (ed. V. Labeyrie, G. Fabres, and D. Lachaise). W. Junk, Dordrecht (1987).

Proceedings of the 7th International Symposium, Insects–Plants, Budapest, 1989 (ed. Á. Szentesi and T. Jermy). *Symposia Biologica Hungarica*, **39**, 1–577 (1991).

Proceedings of the 8th International Symposium, Insect–Plant Relationships, Wageningen, 1992 (ed. S.B.J. Menken, J.H. Visser and P. Harrewijn). Kluwer Academic Publishers, Dordrecht (1992).

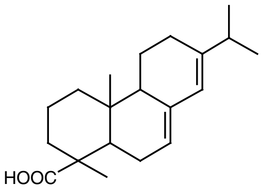
Proceedings of the 9th International Symposium, Insect–Plant Relationships, Gwatt, 1995 (ed. E. Städler, M. Rowell-Rahier, and R. Baur). *Entomologia Experimentalis et Applicata*, **80**, 1–324 (1996).

Proceedings of the 10th International Symposium, Insect–Plant Relationships, Oxford, 1998 (ed. S.J. Simpson, A.J. Mordue (Luntz), and J. Hardie). *Entomologia Experimentalis et Applicata*, **91**, 1–287 (1999).

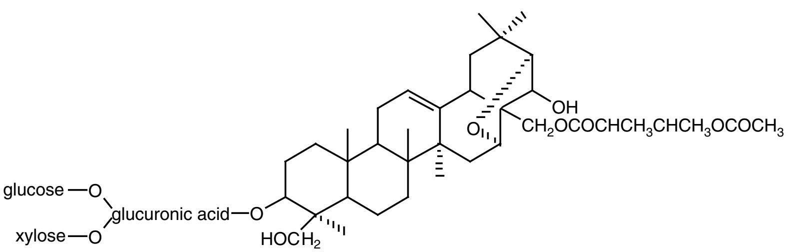
Proceedings of the 11th International Symposium, Insect–Plant Relationships, Helsingør, 2001 (ed. J.K. Nielsen, C. Kjaer, and L.M. Schoonhoven). *Entomologia Experimentalis et Applicata*, **104**, 1–240 (2002).

Proceedings of the 12th International Symposium, Insect–Plant Relationships, Berlin, 2004 (ed. M. Hilker and T. Meiners). *Entomologia Experimentalis et Applicata*, **115**, 1–281 (2005).

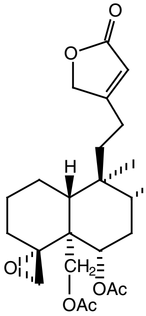
Appendix B: Structural formulae of selected secondary plant compounds



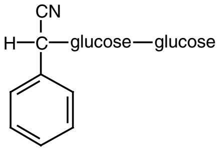
(**1**) Abietic acid



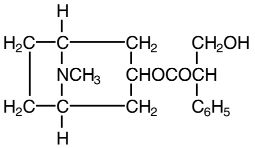
(**2**) Aescin



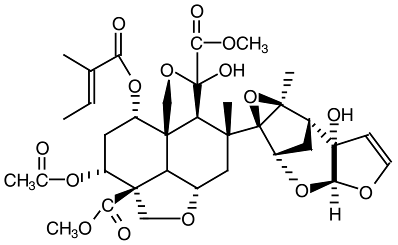
(**3**) Ajugarin I



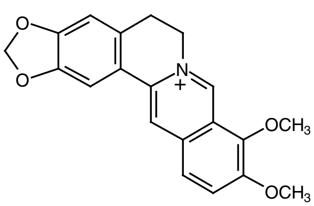
(**4**) Amygdalin



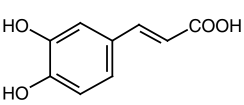
(**5**) Atropine



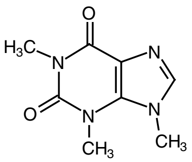
(**6**) Azadirachtin



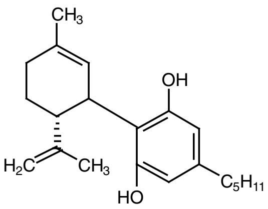
(**7**) Berberine



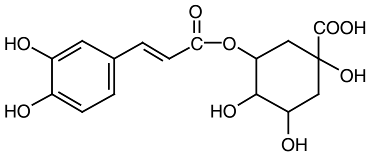
(**8**) Caffeic acid



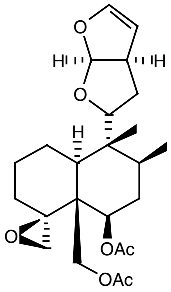
(**9**) Caffeine



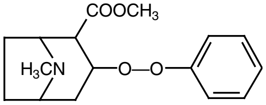
(**10**) Cannabidiol



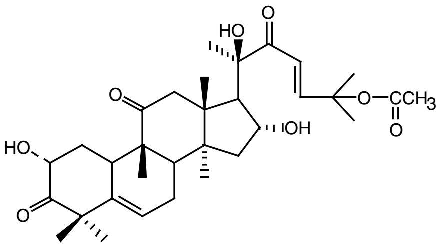
(**11**) Chlorogenic acid



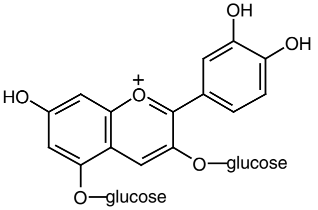
(**12**) Clerodin



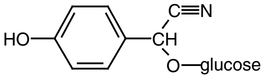
(**13**) Cocaine



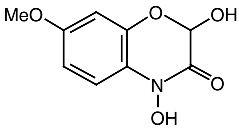
(**14**) Cucurbitacin B



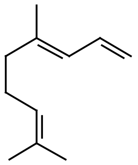
(**15**) Cyanin



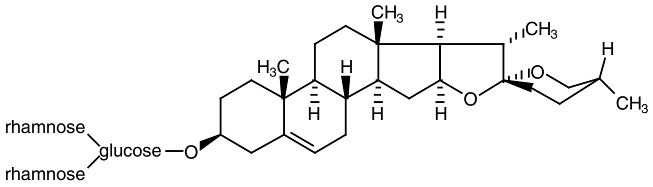
(**16**) Dhurrin



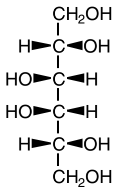
(**17**) DIMBOA



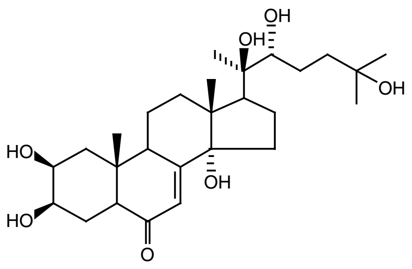
(**18**) 4,8-Dimethyl-1,3(*E*), 7-nonatriene



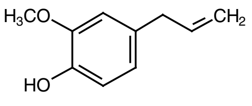
(**19**) Dioscin



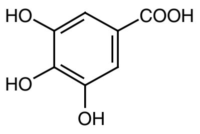
(**20**) Dulcitol



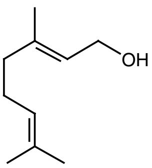
(**21**) β-Ecdyson

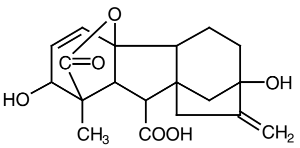


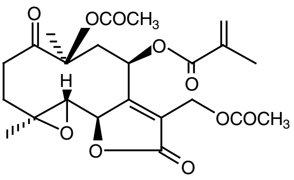
(**22**) Eugenol

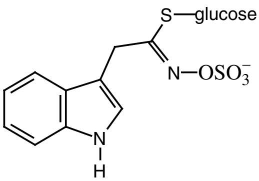


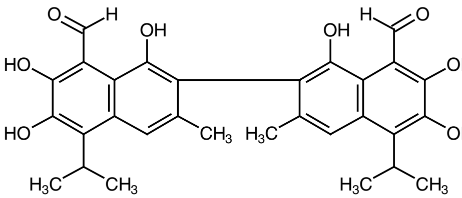
(**23**) Gallic acid

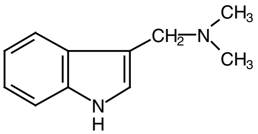

(**24**) Geraniol

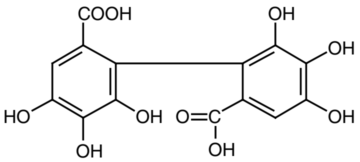

(**25**) Gibberellic acid

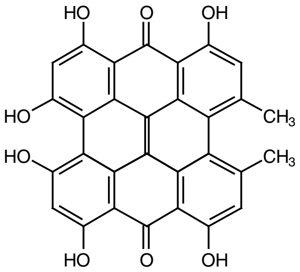

(**26**) Glaucolide A

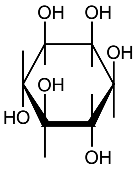

(**27**) Glucobrassicin

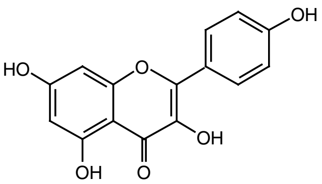

(**28**) Gossypol

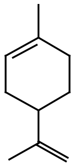

(**29**) Gramine


(**30**) Hexahydroxydiphenic acid


(**31**) Hypericin

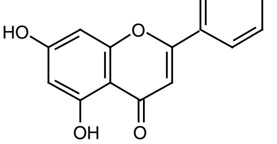

(**32**) Inositol

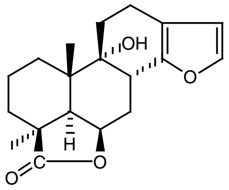

(**33**) Kaempferol

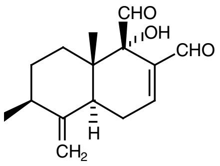

(**34**) Limonene

$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$

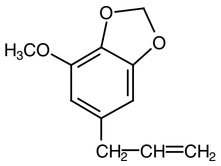
(**35**) Linolenic acid


(**36**) Luteolin

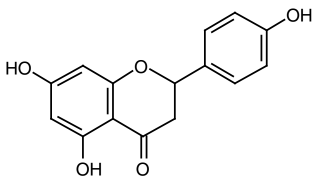

(**37**) Marrubiin



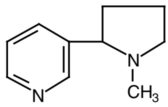
(**38**) Muzigadial



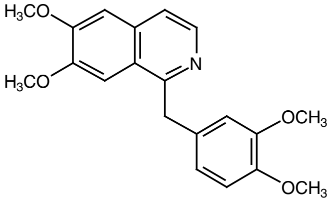
(**39**) Myristicin



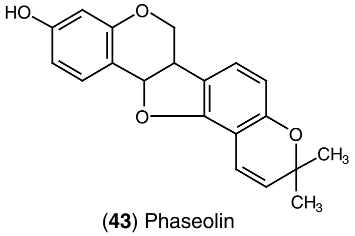
(**40**) Naringenin



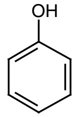
(**41**) Nicotine



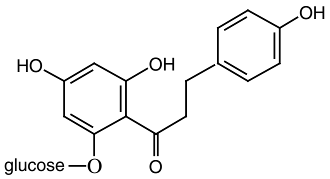
(**42**) Papaverine



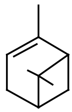
(**43**) Phaseolin



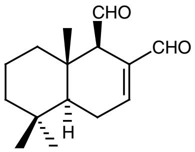
(**44**) Phenol



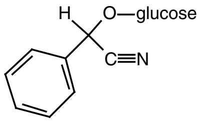
(**45**) Phloridzin



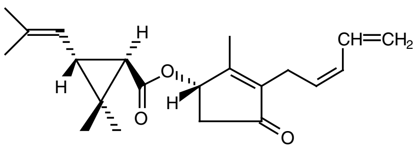
(**46**) α-Pinene



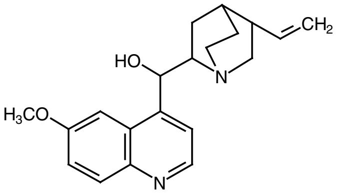
(**47**) Polygodial



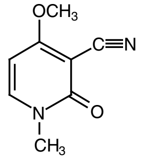
(**48**) Prunasin



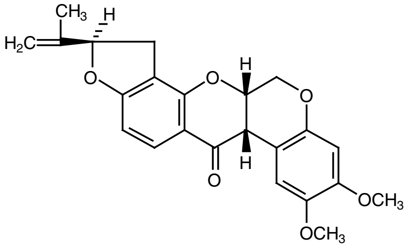
(**49**) Pyrethrin I



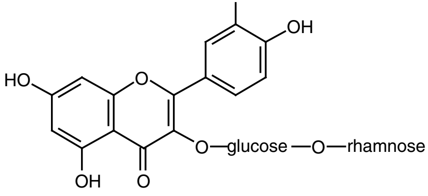
(**50**) Quinine



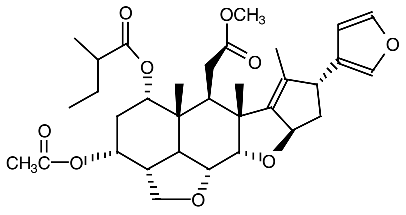
(**51**) Ricinine



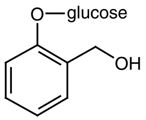
(**52**) Rotenone



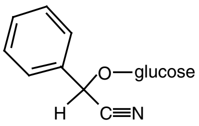
(**53**) Rutin



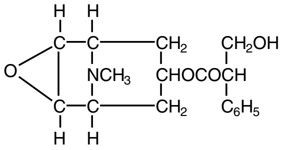
(**54**) Salannin



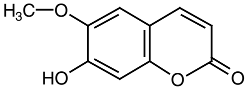
(**55**) Salicin



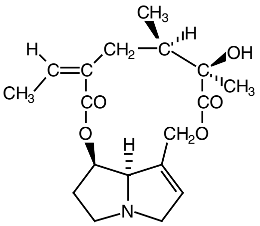
(**56**) Sambunigrin



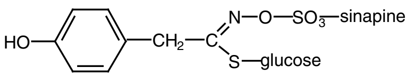
(**57**) Scopolamine



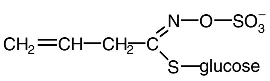
(**58**) Scopoletin



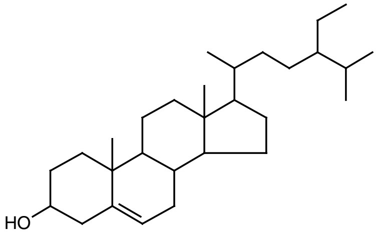
(**59**) Senecionine



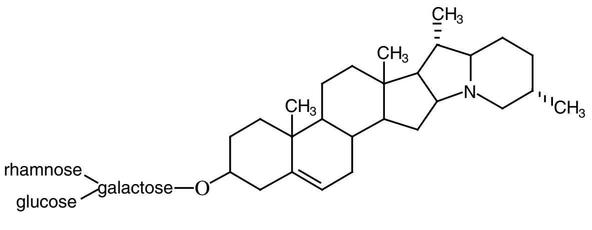
(**60**) Sinalbin



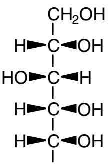
(**61**) Sinigrin



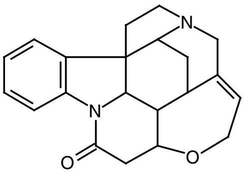
(**62**) Sitosterol



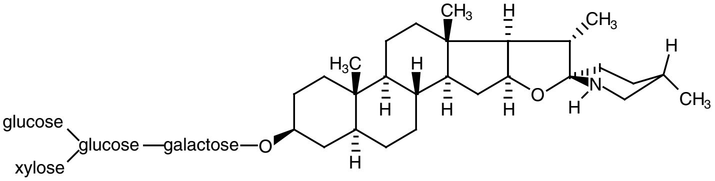
(**63**) α-Solanine



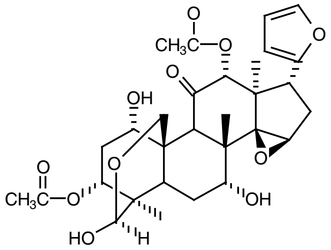
(**64**) Sorbitol



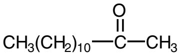
(**65**) Strychnine



(**66**) Tomatine

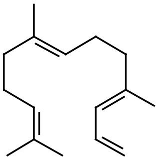


(**67**) Toosendanin

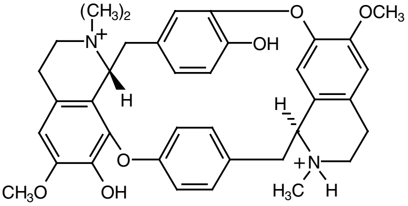


$CH_3(CH_2)_{10}-\overset{O}{\overset{\|}{C}}-CH_3$

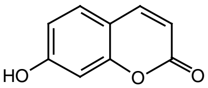
(**68**) 2-Tridecanone



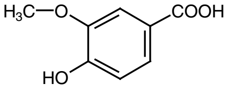
(**69**) 4,8,12-Trimethyl-1,3(*E*), 7(*E*),1-tridecatetraene



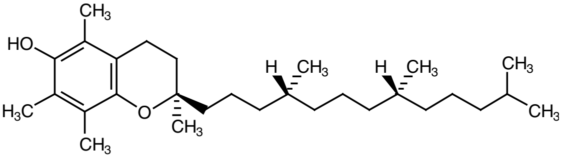
(**70**) Tubocurarine



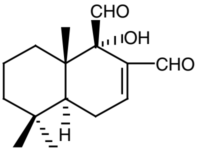
(**71**) Umbelliferone



(**72**) Vanillic acid



(**73**) Vitamin E



(**74**) Warburganal

Appendix C: Methodology

Because each relationship between an insect species and its host plant has unique aspects, its scientific analysis commonly requires the adaptation of existing standard test procedures. This section is intended to list concisely a number of procedures that are often used in the study of insect–plant relationships but generally need to be modified to fit a particular case. For more details the reader is referred to the comprehensive reviews by Miller and Miller,[78] Smith *et al.*,[118] and Hare.[43] Most techniques discussed in this section refer to laboratory studies. Methods for behavioural and experimental studies under field conditions can be found in Miller and Miller[78] and Dent and Walton.[24]

As in other areas of biology the reductionistic approach causes a dilemma: often the most sharply defined set of experimental conditions gives the clearest answer, but such an experimental setting is at the same time most distant from the natural situation that one is attempting to understand. This difficulty can be (partly) circumvented by combining the results from different experimental approaches. Moreover, novel combinations of a reductionistic approach with a functional analysis are emerging, such as the use of carefully characterized genotypes generated through, for example, gene silencing or mutant characterization that are exposed to natural conditions to analyse the consequences of the genetic changes.[26,58]

The pivotal role of plant chemicals in host choice by herbivorous insects is also reflected in this methodology section, which centres on the identification of factors important in host recognition.

C.1 Choice of plants and insects

C.1.1 Plants

Whole plants growing in their natural environment are the ideal material for studying insect responses. Because this is often impractical, or even impossible, potted plants grown in greenhouses can be used as substitutes, although greenhouse plants generally differ substantially from conspecifics grown in the open.[41] Even when plants growing in the open are enclosed in a cage, their physiology may change markedly and with it their nutritional value for insects.[123] The responses of small organisms such as insects can often be studied conveniently in the laboratory by employing plant parts. Although in many cases reliable results will be obtained, plant damage undoubtedly affects the plant's physiology, resulting in changes in its chemical composition. Polyphagous Bertha armyworms, for instance, develop faster on intact tissues of all their host plant species than they do on excised tissues.[28] Undoubtedly wounding effects (see Chapter 4) also play a role. Instances are known in which an insect, when offered leaf discs from different plant species, has shown a reversed preference order to that observed in tests with intact plants.[5,103] Occasionally insects, when kept on the excised leaves of a normally adequate host plant, show considerably increased mortality rates, indicating undesirable changes in chemical composition or moisture content of the food as a result of leaf excision.[86]

Sometimes, noticeable differences exist in the acceptability of leaf discs and excised intact leaves. Young larvae of the leaf beetle *Phyllotreta nemorum* may initiate leaf mining on leaf discs of some non-host plant species, whereas whole leaves of the same plants have proved to be totally unacceptable.[83] It is well known that within hours (and probably sooner) of excision leaves undergo biochemical degradation and changes in water relationships.[16,148] In some cases turgidity can be maintained for long periods by inserting leaf petioles into potato-agar-filled glass vials[136] or by applying water pressure to the cut ends of stems and twigs.[57]

Clip cages are often used to confine small insects to particular sites on a plant in order to measure, for instance, the insect's growth and reproduction. Such cages, however, may exert enough pressure on the leaf surface or affect phyllosphere microclimate to produce physiological and developmental changes in the plant.[21,80]

The best way to investigate the effect of a certain plant characteristic is to compare the response of an insect towards two plant types that are identical except for the characteristic of interest. This was virtually impossible until recently. Well characterized mutants or plants are now available in which certain genes have been knocked out by modern molecular techniques. As a result, highly refined comparisons can be made in the investigation of insect–plant interactions.[104,125,135]

C.1.2 Insects

The principal sources of test insects are laboratory colonies or field-collected material. Although the use of laboratory-reared insects is often more convenient, there is a risk that they differ so radically from natural populations in genetic, behavioural, and physiological characteristics as to limit their representativeness of the species in the wild.[127] Thus, laboratory insects have been reported to lose their ability to grow successfully on their original host plants[40] or have been found to accept plant species totally outside their natural host range.[111] However, field-collected insects may be infested with pathogens and/or parasitoids that strongly affect behaviour compared with non-infected individuals.

An insect's feeding history may also markedly influence its behavioural and physiological responses to normal food plants through preference induction.[53] Occasionally, the induction is so rigid that the insect will die from starvation rather than accept one of its other food-plant species.[42] Clearly, host-plant selection in naive insects may differ markedly from that in experienced insects (see Chapter 8).

A generally neglected aspect of test insects is standardization. As insects collected at different sites or cultured under different conditions may differ greatly in behavioural and physiological characteristics, it is essential for reproducibility of the results and comparison with other studies that the source of the experimental animals is carefully recorded. Studies indicating that different strains of an insect species may perform differently on various natural and semi-synthetic diets have frequently been reported.[18,64]

C.2 Behaviour

The techniques used for analysis of insect behaviour are either direct observation or automatic recording and storage followed by retrieval and analysis. Direct observation procedures are facilitated by employing hand-operated event recorders.[84] Automatic techniques for recording insect behaviour include cinematography and video-recording,[44,61,92] the use of actographs,[4,8] and methods based on electrical registration of feeding activity.[15,129] EthoVision® is an integrated video tracking system for automation of behavioural experiments.[85] There is also a three-dimensional variant of EthoVision®. Activity patterns of insects inside plant tissues or underground may be monitored by acoustic systems.[70]

Experiments in general, but experiments on behaviour in particular, need a thorough consideration of methodology even at the planning stage. Otherwise, an adequate statistical evaluation of the results may become impossible.[76] Statistical evaluation of olfactometer assays[48,108] and food-choice experiments may present special problems, which have been dealt with by several authors.[9,51,66,71]

C.2.1 Olfactory orientation

Many techniques have been developed for studying olfactory responses to plant volatiles.[36] Methods used to investigate insect orientation to odours vary with insect size and type of locomotion (walking or flight). Various methods for collecting and analysing search tracks are reviewed by Bell.[7] Different methods for measuring the responsiveness of an insect to odours may give different results.[124,128] As a consequence, the use of more than one method may provide additional information.

(a) Screen test

A very simple test for walking insects, for instance caterpillars, employs a screen between the plant material and the insect.[25] Direct observation of the insect's behaviour or the distribution of insects after some time provides information on the role of olfactory cues.[19] A modified type of screen test has been used to observe caterpillar reactions to attractive and repellent odours released, for instance, by artificial diets.[109]

(b) Olfactometer tests

In many cases Y-tube olfactometers have proved to be relatively simple yet very useful pieces of apparatus, providing the test insect with a binary choice.[62] Walking as well as flying insects can be tested for their preference for, for instance, an air stream bearing an odour, which passes through one arm of the Y, or for clean air (control), which passes through the other arm. Dimensions and special modifications, for instance a guiding rail for some walking insects, have to be made, according to the size and habits of the insect. A dual-choice arena has proved to be an adequate instrument for testing the responses of groups of moths to plant odours.[82]

(c) Multi-arm olfactometers

A four-arm airflow olfactometer has been designed for small walking insects such as hymenopterous parasitoids.[137] It allows testing of more than one odour or different concentrations of one odour at the same time. In a central arena the insect can choose between four different odour fields. A six-arm olfactometer permits simultaneous testing of six odours or odour concentrations for their relative attractiveness.[132] Special statistical tests have been developed to analyse results obtained with multi-arm olfactometers when simultaneously testing more than two different stimuli or odour concentrations.[132,137]

(d) Wind tunnel

Basically, a wind tunnel consists of three parts: (1) an effuser or entrance zone in which the air is accelerated and the flow is 'smoothed', (2) a working section where the insects are observed, and (3) a diffuser or exhaust zone where the air is decelerated.[141] Walking[138] or flying[91] insects are released in the centre or at the downwind end of the tunnel. Various parameters of an insect's response to air streams with or without plant volatiles are recorded. Useful information on planning wind-tunnel experiments is given by Finch.[36] The risks of pseudo-replications in experiments employing wind tunnels or olfactometers are discussed by Ramírez *et al.*[97]

(e) Locomotion compensator

The locomotion compensator, or 'Kramer sphere', is a sophisticated instrument that permits accurate measurement of orientational responses to windborne volatiles.[63] The test insect sits on a sphere and every displacement is compensated for by a computer-controlled movement of the sphere in the opposite direction. As a result the freely walking insect remains in the same place and its stimulus situation remains constant. The compensator can be operated in combination with visual stimuli if required, and has been used successfully with a wind tunnel to record various locomotion parameters in different types of walking insect. All recorded movements of the sphere allow automatic data analysis.[14,126,128]

(f) Automatic flight recording

A computer-controlled video system for real-time recording of insect flight in three dimensions has been described, and allows analysis of the flight paths of moths in a wind tunnel.[32]

(g) Semi-field set-ups

Making behavioural observations of insects in the field can be quite difficult owing to the small body size of insects, and their mobility and speed. Therefore, as a step between fully conditioned laboratory investigations and field experiments, semi-field set-ups in a laboratory or greenhouse can be performed. For instance, a limited number of plants can be offered to an insect in an area where the insect is free to decide to stay in the set-up or to move away. This allows the observer to make detailed behavioural observations[147] or to investigate the outcome of a behavioural sequence.[27,88]

(h) Tracking insects in the field

Observing insects in the field is not an easy thing to do. Tracking insect movement under field conditions will reveal possible patterns in their searching behaviour under ecologically relevant conditions. Many methods have been used, ranging from mark–release–recapture experiments[133] to observing insects with binoculars.[143] Electronic devices are continuously being miniaturized further, so that they can be attached to insects for tracking in the field. For example, a novel radar-based method has been developed that allows honeybees to be tracked over larger distances by attaching a 16-mm dipole to them. The weight of the dipole was either 0.8 or 12 mg, and bee behaviour such as ground speed, distance from the hive, and maximum range was recorded in the field.[17]

C.2.2 Feeding

The fine details of host recognition are undoubtedly under the control of the insect's contact chemical senses. Taste plays a major role in host-plant choice (see Chapter 7), and choice experiments are a simple and indispensable tool in any insect–plant study. Bioassays employing whole plants or plant parts, for instance leaf discs, may be of the no-choice type, or the insects may be offered a choice between two or more alternatives. Choice tests with more than two alternatives, however, should be avoided, because the results may be ambiguous and difficult to analyse.[94] Both no-choice and binary-choice designs are suitable for answering different questions. The no-choice situation is generally more representative of the field situation in our agricultural systems, and also of natural vegetation where a choice, for example between the leaves of two plant species within a distance of a few millimetres, as in the choice test, is almost never presented. However, binary-choice experiments are often much more sensitive when chemicals are screened for behavioural activity. The polyphagous peach aphid *Myzus persicae*, for instance, readily accepts antifeedant-treated host leaves or artificial foods containing various allelochemicals, but in a choice situation is often seen clearly to prefer the control lacking the test substance.[92,113] Statistical evaluation of choice experiments may present special problems, which have been considered by several authors.[9,51,71]

The role of feeding and oviposition stimulants or deterrents can also be tested by infusing them into the plant tissue. Insects can be exposed to excised non-host plant material with their stems or petioles in a stimulant solution[41] or to host plant material that has taken up a deterrent solution.[12]

(a) Leaf discs

Many test designs are based on the use of leaf discs, mainly to standardize the area of foliage exposed to the insect. A commonly used layout is the 'cafeteria test', in which discs punched out of the leaves of two plant species are offered to the insect in a circular array.[55] When pure compounds are to be tested, discs of host plants can be used as a substrate, for instance to test the efficacy of antifeedants. The experimental discs are either dipped into a solution of the test chemical or the compound is applied with a brush or by spraying. The chemical may also be incorporated into an agar or gelatin cover,[149] or leaf discs (or whole leaves) may be vacuum-infiltrated.[114] To determine whether a plant is not eaten because of the absence of phagostimulants or because it contains feeding deterrents, tests with 'sandwiches' of leaf discs from the test plant combined with those of a host plant may provide the answer.[52]

When leaf discs are used to determine the activity of an antifeedant, the choice of the plant species may affect the insect's sensitivity to the antifeedant compound. The feeding deterrence of azadirachtin, for instance, to *Spodoptera frugiperda* larvae was much higher on cotton leaf discs than on lima bean.[93] Ingestion of leaf discs or neutral substrates can be measured manually (weight, surface area consumed) or automatically.[33,87] It is important that studies of herbivore consumption report the thickness, density, and specific leaf weight of test leaves, and in addition provide at least two measures of consumption (leaf area and biomass removed),[144] because during the short period (hours) for which leaf-disc experiments normally last the herbivore is mostly using volumetric regulation of meal size.[115]

(b) Neutral substrates

Rather than using leaf material as a substrate, as this may introduce unwanted sources of variation, neutral substrates may be employed to test responses to particular chemicals. Thus elderberry pith, filter paper, and glass-fibre discs have often been used for locusts[11] and caterpillars.[121] Styropor lamellae[3] and agar or agar–cellulose blocks[68] have also been found to be useful. It should be realized that chemicals applied to neutral substrates are not necessarily distributed evenly.[150] When testing antifeedant compounds the neutral substrate must be made palatable, usually with sucrose. Several non-nutritional insect phagostimulants may also be useful, and can be obtained commercially.[67] Ingestion in no-choice experiments can also be determined on the basis of the dry weight of faeces produced.[13,56]

(c) Fluid diets

A number of insect species with piercing–sucking mouthparts will feed on artificial diets contained in a Parafilm® sachet.[6,79] The rate of ingestion[130] and effects of phagostimulant or antifeedant compounds can be quantified by adding them to the diet,[113,119] or the test chemical can be painted on the Parafilm® membrane. It is impossible to assess visually when piercing insects such as aphids,[90,129,77] thrips,[45,60] leaf-hoppers,[59] and plant bugs[20] are feeding. An electronic method, the electrical penetration graph (EPG), has been developed, which signals various feeding activities once the insect has started to penetrate plant tissues or an artificial diet with its mouthparts.[99,129,134] A computer program for automatic calculation of EPG parameters enables fast processing of the abundant data generated with this method.[34]

Membrane feeding allows light microscopic live observation of stylet movements, during stylet penetration, which is impossible when feeding on plant tissues. Ingestion and salivation activities are visible to some extent as well,[46,75,77] although light microscopy has a low resolution with respect to the stylet dimensions. Therefore, some of the conclusions based on these methods appear to be rather speculative. The drawback of membrane feeding experiments used to investigate chemical and mechanical aspects is that extrapolation of results to the natural situation (i.e. feeding on plants) remains speculative. Experimentally, however, the use of fluid diets often has many advantages over complete plants.

Drinking responses have also been used for a fast assessment of antifeedant effects. In this case the test fluid is administered by a small platinum-wire loop[30] or by a microsyringe to the mouthparts of chewing insects during feeding.[114] In the case of heteropterans micropipettes have been employed to offer drinking water containing test chemicals.[112]

C.2.3 Oviposition

When searching for an oviposition site the females of many herbivorous insect species are guided by a complex of visual, olfactory, contact chemical, shape, and/or tactile cues specific to their host plants. For flying insects, field cage experiments, as a semi-laboratory method,[29] provide conditions that are closest to the natural situation. At present, however, a detailed analysis of oviposition behaviour can be carried out only by laboratory experiments. The set-ups for such tests have to be designed specifically for each insect species. Here only some references can be given as examples of oviposition assay methods for flying insects, such as lepidopterans[50,69,98,131] and flies,[23,81,105] as well as for walking insects.[54]

C.3 Sensory physiology

As insect feeding behaviour is to a large extent governed by chemosensory information, the analysis of sensory responses to plant chemicals may provide important clues to the role of different chemicals in host-plant recognition. The contribution of the chemical senses to the decision-making process can be studied by ablation techniques and electrophysiological methods.

C.3.1 Ablation

The role of specific sensory hairs or organs can be assessed by inactivation or by ablating them and observing changes in the insect's behavioural responses to chemical stimuli. Non-selective inactivation can be done by applying aggressive chemicals, such as hydrochloric acid,[120] to the sensillum or by electrical cauterization.[13] Ablation may be effected by microsurgery.[68]

C.3.2 Electrophysiology

Sensory responses to either pure compounds or mixtures can be recorded from individual olfactory or taste cells by electrophysiological techniques.[37] The extracellularly recorded action potentials have a small amplitude, necessitating the use of amplifiers.[74] Experiments are usually performed on isolated heads or legs, but there is no reason, other than inconvenience caused by movements, why intact insects cannot be used. Water anaesthetization provides a method by which taste recordings can be made from intact caterpillars.[39] As the electrical signals from the sensilla are produced in different neurons, computer programs have been developed to analyse the complex spike patterns obtained with single-cell recordings.[72,117]

Because an insect often possesses many olfactory cells conveniently located on one of the head appendages (i.e. the antennae), electroantennography (EAG) is a useful technique for examining a summated response of the olfactory system.[37,89] EAGs are recorded either from excised antennae or from intact insects. The EAG is thought to reflect the summation of receptor potentials over the whole antenna, and the response amplitude is positively correlated with the number of sensilla housing sensitive receptor cells. When classifying plant volatiles for their capacity to evoke olfactory activity, EAG appears to be a useful technique as it provides a screening of the entire antennal receptor population. EAG does not, however, allow conclusions to be drawn about the specificity of the responding (sub)populations of antennal olfactory cells. Neither does it enable any conclusion to be made regarding behavioural attractiveness or deterrency of the stimulus. EAGs increase with increasing concentration of the chemical stimulus, until a saturation level is reached (see Fig. 6.17).

The EAG technique, which is also applicable to small insects,[96,139] is especially useful in combination with gas chromatography.[73,152] Direct coupling of both techniques allows the identification of volatiles in complex mixtures and simultaneous on-line determination of the biological activity of individual odour components.

Results obtained with the EAG technique may be divergent from those obtained with the single-cell recording method, showing that both have their own merit.[146]

C.4 Plant chemistry

C.4.1 Headspace

Volatiles emitted by intact or insect-damaged plants can be collected on to an absorbent material ('odour trap') or in a cold trap,[107] from which they can later be readily de-absorbed and, after concentration, analysed by gas chromatography and mass spectrometry.[1,10,95]

C.4.2 Leaf surface

Chemicals present on plant surfaces can be extracted by dipping intact plants briefly into organic solvents. As the waxy surface of most plant species contains a mixture of polar and non-polar compounds, solvents must be chosen that dissolve both. Various methods of extraction and identification of chemicals on plant surfaces have been used and are being developed.[31,100,122,142]

C.4.3 Plant interior

A general problem is that most chemicals that play an important role in host selection by insects originate from within living organisms. Once the plant has been prepared in any way for analysis, its metabolic state may have changed and with it the quantity and quality of its allelochemicals. Most extraction procedures start by homogenizing plant parts in a blender in order to crush all cells, causing the release of their contents into the extracting solvent. All soluble chemicals can then be extracted following any of several methods, including those cited by Smith *et al*.[118] When one is interested in a particular group of chemicals, chemical analysis can be focused on the isolation and identification of individual compounds. The numerous and special problems met during the investigation of particular classes of allelochemicals can be found in comprehensive texts, such as Rosenthal and Berenbaum[106] and Waterman and Mole.[145] In recent years analytical methodology has developed enormously so that now many secondary plant compounds can be analysed simultaneously using, for example, liquid chromatograpy–time of flight (LC-TOF) machines.[35] Some methods combine the analysis of phytohormones, phytotoxins, and headspace volatiles.[116] In this way the highly complex chemistry of plants can be accurately investigated through emerging metabolomics technology.

Stylectomy (cutting an aphid's stylets by radiofrequency microcautery during feeding) is an excellent technique for collecting phloem sap to be used for analysis of its chemical composition.[38]

C.4.4 Gene expression patterns

Plants and insects are phenotypically plastic (see Chapters 4 and 8, respectively). This means that the characteristics of each individual in a population are dependent on the internal and external conditions experienced. Experience may influence gene expression patterns, such as the expression of defensive plant genes that may be dependent on induction by herbivory. To find those genes that are expressed in response to herbivory, a differential display procedure may be used. Basically, this is a method that investigates the differences in RNA molecules between plants undergoing different treatments, such as an unwounded control and a herbivore-damaged treatment plant (e.g. Voelckel and Baldwin[140]). By using a real-time polymerase chain reaction (RT-PCR), the relevant DNA sequences may be generated to build a subtractive library for use in subsequent studies aimed at elucidating, for example, temporal, ontogenetic, or spatial differences in the plant response. To investigate how herbivory affects gene expression, northern blot analyses may be performed for genes of interest.[47] However, when more genes of a plant species have been identified, dedicated microarrays can be developed in which the effects of herbivory or other environmental stresses on a large number of genes can be investigated simultaneously.[101,102] Ultimately, if the full genome has been sequenced, a genome-wide effect of environmental stress on plant gene expression can be determined.[110] At present, the full genome has been sequenced for *Arabidopsis thaliana*,[2] but sequence projects are under way for other plant species as well. Moreover, plant species that are closely related to a fully sequenced plant may be analysed through microarray technology. For instance, gene expression patterns in *Brassica* species may be investigated with 70-mer oligo microarrays based on the *Arabidopsis* genome.[65]

With gene expression technology, transcriptome patterns can be investigated in the laboratory as well as the field. This is likely to open exciting new ways of assessing the expressed genotype under field conditions which can then be linked to the expression of the phenotype, that is, the effects on interactions with other organisms in the environment. This new research field, called environmental genomics, is expected signficantly to advance our understanding of the way in which plant characteristics affect interactions with insects.

Similar methods can also be applied to insects. A few insect species have been sequenced so far, including the fruit fly *Drosophila melanogaster* and the malaria mosquito *Anopheles gambiae*.[49] Although this is a good starting point, the sequencing of herbivorous insects will be a major step in the application of transcriptome analysis to the field of insect–plant interactions. In this respect it is exciting that the first genome of a herbivorous insect has recently been sequenced, that of the silkmoth *Bombyx mori*.[151]

C.5 References

1. Agelopoulos, N.G., Hooper, A.M., Maniar, S.P., Pickett, J.A., and Wadhams, L.J. (1999). A novel approach for isolation of volatile chemicals released by individual leaves of a plant *in situ*. *Journal of Chemical Ecology*, **25**, 1411–25.
2. Arabidopsis Genome Project (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature*, **408**, 796–815.
3. Ascher, K.R.S. and Gurevitz, E. (1972). A further of the 'styropor method': evaluating the response of the fruit bark beetle, *Scolytus* (*Ruguloscolytus*) *mediterraneus* Eggers, to extracts of its host plant. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, **79**, 215–22.
4. Ayertey, J.N. (1981). Locomotor activity of *Sitophilus zeamais* and *Sitotroga cerealella* on maize. *Entomologia Experimentalis et Applicata*, **29**, 19–28.
5. Barnes, O.L. (1963). Food plant tests with the differential grasshopper. *Journal of Economic Entomology*, **56**, 396–9.
6. Begum, M.N. and Wilkins, R.M. (1998). A parafilm sachet technique for measuring the feeding of *Nilaparvata lugens* on rice plants with correction for evapotranspiration. *Entomologia Experimentalis et Applicata*, **88**, 301–4.
7. Bell, W.J. (1991). *Searching behaviour. The behavioural ecology of finding resources*. Chapman & Hall, London.
8. Bernays, E.A. (1979). The use of Doppler actographs to measure locomotor activity in locust nymphs. *Entomologia Experimentalis et Applicata*, **26**, 136–41.
9. Bernays, E.A. and Weiss, M.R. (1996). Induced food preferences in caterpillars: the need to identify mechanisms. *Entomologia Experimentalis et Applicata*, **78**, 1–6.
10. Blaakmeer, A., Geervliet, J.B.F., Van Loon, J.J.A., Posthumus, M.A., Van Beek, T.A., and De Groot, Æ. (1994). Comparative headspace analysis of cabbage plants damaged by two species of *Pieris* caterpillars: consequences for in-flight host location by *Cotesia* parasitoids. *Entomologia Experimentalis et Applicata*, **73**, 175–82.
11. Blaney, W.M. and Winstanley, C. (1980). Chemosensory mechanism of locusts in relation to feeding: the role of some secondary plant compounds. In *Insect Neurobiology and Pesticide Action (Neurotox 79)*, pp. 383–9. Chemical Industry, London.
12. Blau, P.A., Feeny, P., Contardo, L., and Robson, D.S. (1978). Allylglucosinolate and herbivorous caterpillars: a contrast in toxicity and tolerance. *Science*, **200**, 1296–8.
13. Blom, F. (1978). Sensory activity and food intake: a study of input–output relationships in two phytophagous insects. *Netherlands Journal of Zoology*, **28**, 277–340.
14. Bolter, C.J., Dicke, M., Van Loon, J.J.A., Visser, J.H., and Posthumus, M.A. (1997). Attraction of Colorado potato beetle to herbivore damaged plants during herbivory and after its termination. *Journal of Chemical Ecology*, **23**, 1003–23.
15. Bowdan, E. (1984). An apparatus for the continuous monitoring of feeding by caterpillars in choice, or non-choice tests (automated cafetaria test). *Entomologia Experimentalis et Applicata*, **36**, 13–16.
16. Bowers, M.D., Stamp, N.E., and Fjaer, E.D. (1991). Factors affecting calculation of nutritional indices for foliage-fed insects: an experimental approach. *Entomologia Experimentalis et Applicata*, **61**, 101–16.
17. Capaldi, E.A., Smith, A.D., Osborne, J.L., Fahrbach, S.E., Farris, S.M., Reynolds, D.R., *et al.* (2000). Ontogeny of orientation flight in the honeybee revealed by harmonic radar. *Nature*, **403**, 537–40.
18. Carpenter, J.E. and Bloem, S. (2002). Interaction between insect strain and artificial diet in diamondback moth development and reproduction. *Entomologia Experimentalis et Applicata*, **102**, 283–94.
19. Chin, C.T. (1950). Studies on the physiological relations between the larvae of *Leptinotarsa decemlineata* Say and some solanaceous plants. *Tijdschrift over Plantenziekten*, **56**, 1–88.
20. Cline, A.R. and Backus, E.A. (2002). Correlations among AC electronic monitoring waveforms, body postures, and stylet penetration behaviors of *Lygus hesperus* (Hemiptera: Miridae). *Environmental Entomology*, **31**, 538–49.
21. Crafts, B.S.J. and Chu, C. (1999). Insect clip cages rapidly alter photosynthetic traits of leaves. *Crop Science*, **39**, 1896–9.
22. Deleted.
23. Degen. T. and Städler, E. (1996). Influence of natural leaf shapes on oviposition in three phytophagous flies: a comparative study. *Entomologia Experimentalis et Applicata*, **80**, 97–100.
24. Dent, D.R. and Walton, M.P. (1997). *Methods in ecological and agricultural entomology*. CABI, Wallingford.
25. Dethier, V.G. (1947). *Chemical insect attractants and repellents*. Blakiston, Philadelphia.

26. Dicke, M. and Hilker, M. (2003). Induced plant defences: from molecular biology to evolutionary ecology. *Basic and Applied Ecology*, **4**, 3–14.
27. Dicke, M., De Boer, J.G., Hofte, M., and Rocha-Granados, M.C. (2003). Mixed blends of herbivore-induced plant volatiles and foraging success of carnivorous arthropods. *Oikos*, **101**, 38–48.
28. Dosdall , L.M. and Ulmer, B.J. (2004). Feeding, development, and oviposition of Bertha armyworm (Lepidoptera: Noctuidae) on different host plant species. *Environmental Entomology*, **33**, 756–64.
29. Duan, J.J. and Prokopy, R.J. (1994). Apple maggot fly response to red sphere traps in relation to fly age and experience. *Entomologia Experimentalis et Applicata*, **73**, 279–87.
30. Eger, H. (1937). Über den Geschmackssinn von Schmetterlingsraupen. *Biologisches Zentralblatt*, **57**, 293–308.
31. Eigenbrode, S.D. and Espelie, K.E. (1995). Effects of plant epicuticular lipids on insect herbivores. *Annual Review of Entomology*, **40**, 171–94.
32. El-Sayed, A.M., Godde, J., and Arn, H. (2000). A computer-controlled video system for real-time recording of insect flight in three dimensions. *Journal of Insect Behavior*, **13**, 881–900.
33. Escoubas, P., Lajide, L., and Mitzutani, J. (1993). An improved leaf-disk antifeedant bioassay and its application for the screening of Hokkaido plants. *Entomologia Experimentalis et Applicata*, **66**, 99–107.
34. Febvay, G., Rahbé, Y., and Van Helden, M. (1996). MacStylet, software to analyse electrical penetration graph data on the Macintosh. *Entomologia Experimentalis et Applicata*, **80**, 105–8.
35. Fiehn, O. (2002). Metabolomics—the link between genotypes and phenotypes. *Plant Molecular Biology*, **48**, 155–71.
36. Finch, S. (1986). Assessing host-plant finding by insects. In *Insect–plant interactions* (ed. J.R. Miller and T.A. Miller), pp. 23–63. Springer, New York.
37. Frazier, J.L. and Hanson, F.E. (1986). Electrophysiological recording and analysis of insect chemosensory reponses. In *Insect–plant interactions* (ed. J.R. Miller and T.A. Miller), pp. 285–330. Springer, New York.
38. Girousse, C., Bonnemain, J.L., Delrot, S., and Bournoville, R. (1991). Sugar and amino acid composition of phloem sap of *Medicago sativa*: a comparative study of two collecting methods. *Plant Physiology and Biochemistry*, **29**, 41–8.
39. Gothilf, S. and Hanson, F.E. (1994). A technique for electrophysiologically recording from chemosensory organs of intact caterpillars. *Entomologia Experimentalis et Applicata*, **72**, 305–10.
40. Guthrie, W.D. and Carter, S.W. (1972). Backcrossing to increase survival of larvae of a laboratory culture of the European corn borer on field corn. *Annals of the Entomological Society of America*, **65**, 108–9.
41. Hammond, R.B., Pedigo, L.P., and Poston, F.L. (1979). Green cloverworm leaf consumption on greenhouse and field soybean leaves and development of a leaf-consumption model. *Journal of Economic Entomolology*, **72**, 714–17.
42. Hanson, F.E. (1976). Comparative studies on induction of food preferences in lepidopterous larvae. *Symposia Biologica Hungarica*, **16**, 71–7.
43. Hare, J.D. (1998). Bioassay methods with terrestrial invertebrates. In *Methods in chemical ecology*, Vol. 2 (ed. K.F. Haynes and J.G. Millar), pp. 212–70. Chapman & Hall, New York.
44. Hargrove, W.W. and Crossley, D.A. (1988). Video digitizer for the rapid measurement of leaf area lost to herbivorous insects. *Annals of the Entmological Society of America*, **81**, 593–8.
45. Harrewijn, P., Tjallingii, W.F., and Mollema, C. (1996). Electrical recording of plant penetration by western flower thrips. *Entomologia Experimentalis et Applicata*, **79**, 345–54.
46. Harris, K.F. and Bath, J.E. (1973). Regurgitation by *Myzus persicae* (Homoptera: Aphididae) during membrane feeding: its likely function in transmission of nonpersistent plant viruses. *Annals of the Entomological Society of America*, **66**, 793–6.
47. Hermsmeier, D., Schittko, U., and Baldwin, I.T. (2001). Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. I. Large-scale changes in the accumulation of growth- and defense-related plant mRNAs. *Plant Physiology*, **125**, 683–700.
48. Hern, A. and Dorn, S. (2001). Statistical modelling of insect behavioural responses in relation to the chemical composition of test extracts. *Physiological Entomology*, **26**, 381–90.
49. Holt, R.A., Subramanian, G.M., Halpern, A., Sutton, G.G., Charlab, R., Nusskern, D.R., *et al*. (2002). The genome sequence of the malaria mosquito *Anopheles gambiae*. *Science*, **298**, 129–49.
50. Hora, K.H. and Roessingh, P. (1999). A novel bioassay for *Yponomeuta cagnagellus* oviposition in response to extracts of host and nonhost plant surface compounds. *Journal of Chemical Ecology*, **25**, 2547–59.
51. Horton, D.R. (1995). Statistical considerations in the design and analysis of paired-choice assays. *Environmental Entomology*, **24**, 179–91.
52. Jermy, T. (1966). Feeding inhibitors and food preference in chewing phytophagous insects. *Entomologia Experimentalis et Applicata*, **9**, 1–12.

53. Jermy, T. (1987). The role of experience in the host selection of phytophagous insects. In *Perspectives in chemoreception and behavior* (ed. R.F. Chapman, E.A. Bernays, and J.G. Stoffolano), pp. 142–57. Springer, New York.
54. Jermy, T. and Szentesi, Á. (1978). The role of inhibitory stimuli in the choice of oviposition site by phytophagous insects. *Entomologia Experimentalis et Applicata*, **24**, 458–71.
55. Jermy, T., Hanson, F., and Dethier, V.G. (1968). Induction of specific food preference in lepidopterous larvae. *Entomologia Experimentalis et Applicata*, **11**, 211–30.
56. Kasting, R. and McGinnis, A.J. (1962). Quantitative relationships between consumption and excretion of dry matter by larvae of the pale western cutworm, *Agrotis orthogonia* Morr. (Lepidoptera: Noctuidae). *Canadian Entomologist*, **94**, 441–3.
57. Kendall, R.D. (1957). Simple devices for keeping foodplants fresh. *Lepidopterists News*, **11**, 225–6.
58. Kessler, A., Halitschke, R., and Baldwin, I.T. (2004). Silencing the jasmonate cascade: induced plant defenses and insect populations. *Science*, **305**, 665–8.
59. Kimmins, F. and Bosque-Perez, N. (1996). Electrical penetration graphs from *Cicadulina* spp. and the inoculation of a persistent virus into maize. *Entomologia Experimentalis et Applicata*, **80**, 45–9.
60. Kindt, F., Joosten, N.N., Peters, D., and Tjallingii, W.F. (2003). Characterisation of the feeding behaviour of western flower thrips in terms of electrical penetration graph (EPG) waveforms. *Journal of Insect Physiology*, **49**, 183–91.
61. Kindvall, O., Nordlander, G., and Nordenhem, H. (2000). Movement behaviour of the pine weevil *Hylobius abietis* in relation to soil type: an arena experiment. *Entomologia Experimentalis et Applicata*, **95**, 53–61.
62. Koschier, E.H., De Kogel, W.J., and Visser, J.H. (2000). Assessing the attractiveness of volatile plant compounds to western flower thrips *Frankliniella occidentalis. Journal of Chemical Ecology*, **26**, 2643–55.
63. Kramer, E. (1976). The orientation of walking honeybees in odour fields with small concentration gradients. *Physiological Entomology*, **1**, 27–37.
64. Lecomte, C., Pierre, D., Pouzat, J., and Thibout, E. (1998). Behavioural and olfactory variations in the leek moth, *Acrolepiopsis assectella*, after several generations of rearing under diverse conditions. *Entomologia Experimentalis et Applicata*, **86**, 305–11.
65. Lee, H.S., Wang, J.L., Tian, L., Jiang, H.M., Black, M.A., Madlung, A., *et al*. (2004). Sensitivity of 70-mer oligonucleotides and cDNAs for microarray analysis of gene expression in *Arabidopsis* and its related species. *Plant Biotechnology Journal*, **2**, 45–57.
66. Lockwood III, J.R. (1998). On the statistical analysis of multiple-choice feeding preference experiments. *Oecologia*, **116**, 475–81.
67. Lopez, J.D. and Lingren, P.D. (1994). Feeding response of adult *Helicoverpa zea* (Lepidoptera: Noctuidae) to commercial phagostimulants. *Journal of Economic Entomology*, **87**, 1653–8.
68. Ma, W.C. (1972). Dynamics of feeding responses in *Pieris brassicae* (Linn) as a function of chemosensory input: a behavioural, ultrastructural and electrophysiological study. *Mededelingen Landbouwhogeschool Wageningen*, **72-11**, 1–162.
69. Ma, W.C. and Schoonhoven, L.M. (1973). Tarsal contact chemosensory hairs of the large white butterfly *Pieris brassicae* and their possible role in oviposition behaviour. *Entomologia Experimentalis et Applicata*, **16**, 343–57.
70. Mankin, R.W., Brandhorst-Hubbard, J., Flanders, K.L., Zhang, M., Crocker, R.L., Lapointe S.L., *et al*. (2000). Eavesdropping on insects hidden in soil and interior structures of plants. *Journal of Economic Entomology*, **93**, 1173–82.
71. Manly, B.F.J. (1995). Measuring selectivity from multiple choice feeding-preference experiments. *Biometrics*, **51**, 709–15.
72. Marion-Poll, F. (1996). Display and analysis of electrophysiological data under Windows^{TM}. *Entomologia Experimentalis et Applicata*, **80**, 116–19.
73. Marion-Poll, F. and Thiéry, D. (1996). Dynamics of EAG responses to host-plant volatiles delivered by a gas chromatograph. *Entomologia Experimentalis et Applicata*, **80**, 120–3.
74. Marion-Poll, F. and Van der Pers, J. (1996). Un-filtered recordings from insect taste sensilla. *Entomologia Experimentalis et Applicata*, **80**, 113–15.
75. Martin, B., Collar, J.L., Tjallingii, W.F., and Fereres, A. (1997). Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses. *Journal of General Virology*, **78**, 2701–5.
76. Martin, P. and Bateson, P. (1986). *Measuring behaviour. An introductory guide*. Cambridge University Press, Cambridge.
77. McLean, D.L. and Kinsey, M.G. (1965). Identification of electrically recorded curve patterns associated with aphid salivation and ingestion. *Nature*, **205**, 1130–1.
78. Miller, J.R. and Miller, T.A. (1986). *Insect–plant interactions*. Springer, New York.
79. Mittler, T.E. (1988). Application of artificial feeding techniques for aphids. In *Aphids, their biology, natural*

enemies and control, Vol. 2B (ed. A.K. Minks and P. Harrewijn), pp. 145–70. Elsevier, Amsterdam.

80. Moore, J.P., Taylor, J.E., Paul, N.D., and Whittaker, J.B. (2003). The use of clip cages to restrain insects reduces leaf expansion systemically in *Rumex obtusifolius. Ecological Entomology*, **28**, 239–42.
81. Nair, K.S.S., McEwen, F.L., and Snieckus, V. (1976). The relationship between glucosinolate content of cruciferous plants and oviposition preferences of *Hylemya brassicae* (Diptera: Anthomyidae). *Canadian Entomologist*, **108**, 1031–6.
82. Natale, D., Mattiacci, L., Hern, A., Pasqualini, E., and Dorn, S. (2004). Bioassay approaches to observing behavioural responses of adult female *Cydia molesta* to host plant odour. *Journal of Applied Entomology*, **128**, 182–7.
83. Nielsen, J.K. (1989). The effect of glucosinolates on reponses of young *Phyllotreta nemorum* larvae to non-host plants. *Entomologia Experimentalis et Applicata*, **51**, 249–59.
84. Noldus, L.P.J.J. (1991). The Observer. A software system for collection and analysis of observational data. *Behavior Research Methods and Instrumentation*, **23**, 415–29.
85. Noldus, L.P.J.J., Spink, A.J., and Tegelenbosch, R.A.J. (2001). EthoVision: a versatile video tracking system for automation of behavioral experiments. *Behavior Research Methods, Instruments, & Computers*, **33**, 398–414.
86. Olckers, T. and Hulley, P.E. (1994). Host specificity tests on leaf-feeding insects: aberrations from the use of excised leaves. *African Entomology*, **2**, 68–70.
87. O'Neal, M.E., Landis, D.A., and Isaacs, R. (2002). An inexpensive, accurate method for measuring leaf area and defoliation through digital image analysis. *Journal of Economic Entomology*, **95**, 1190–4.
88. Pallini, A., Janssen, A., and Sabelis, M.W. (1999). Spider mites avoid plants with predators. *Experimental and Applied Acarology*, **23**, 803–15.
89. Park, K.C. and Baker, T.C. (2002). Improvement of signal-to-noise ratio in electroantennogram responses using multiple insect antennae. *Journal of Insect Physiology*, **48**, 1139–45.
90. Park, K.C. and Hardie, J. (1998). An improved aphid electroantennogram. *Journal of Insect Physiology*, **44**, 919–28.
91. Phelan, P.L., Roelofs, C.J., Youngman, R.R., and Baker, T.C. (1991). Characterization of chemicals mediating ovipositional host-plant finding by *Amyelois transitella* females. *Journal of Chemical Ecology*, **17**, 599–613.
92. Powell, G., Hardie, J., and Pickett, J.A. (1995). Responses of *Myzus persicae* to the repellent polygodial in choice and no-choice video assays with young and mature leaf tissue. *Entomologia Experimentalis et Applicata*, **74**, 91–5.
93. Raffa, K.F. (1987). Influence of host plant on deterrence by azadirachtin of feeding by fall armyworm larvae (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, **80**, 384–7.
94. Raffa, K.F., Havill, N.P., and Nordheim, E.V. (2002). How many choices can your test animal compare effectively? Evaluating a critical assumption of behavioral preference tests. *Oecologi*a, **133**, 422–9.
95. Raguso, R.A. and Pellmyr, O. (1998). Dynamic headspace analysis of floral volatiles: a comparison of methods. *Oikos*, **81**, 238–54.
96. Ramachandran, R. and Norris, D.M. (1991). Volatiles mediating plant–herbivore–natural-enemy interactions: electroantennogram responses of soybean looper, *Pseudoplusia includens* and a parasitoid, *Microplitis demolitor*, to green leaf volatiles. *Journal of Chemical Ecology*, **17**, 1665–90.
97. Ramírez, C.C., Fuentes-Contreras, E., Rodríguez, L.C., and Niemeyer, H.M. (2000). Pseudoreplication and its frequency in olfactometric laboratory studies. *Journal of Chemical Ecology*, **26**, 1423–31.
98. Reed, D.W., Pivnick, K.A., and Underhill, E.W. (1989). Identification of chemical oviposition stimulants for the diamond-back moth, *Plutella xylostella*, present in three species of Brassicacea. *Entomologia Experimentalis et Applicata*, **65**, 267–82.
99. Reese, J.C., Tjallingii, W.F., Van Helden, M., and Prado, E. (2001). Waveform comparison among AC and DC electronic monitoring systems for aphid (Homoptera: Aphididae) feeding behavior. In *Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior* (ed. G.P. Walker and E.A. Backus), pp. 70–104. Thomas Say Publications in Entomology, Proceedings of the Symposium at the XIX International Congress of Entomology, Beijing, China.
100. Reifenrath, K., Riederer, M., and Müller, C. (2005). Leaf surface wax layers of Brassicaceae lack feeding stimulants for *Phaedon cochleariae. Entomologia Experimentalis et Applicata*, **115**, 41–50.
101. Reymond, P., Weber, H., Damond, M., and Farmer, E.E. (2000). Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis. Plant Cell*, **12**, 707–19.
102. Reymond, P., Bodenhausen, N., Van Poecke, R.M.P., Krishnamurthy, V., Dicke, M., and Farmer, E.E. (2004). A conserved transcript pattern in response to a specialist and a generalist herbivore. *Plant Cell*, **16**, 3132–47.

103. Risch, S.J. (1985). Effects of induced chemical changes on interpretation of feeding preference tests. *Entomologia Experimentalis et Applicata*, **39**, 81–4.
104. Roda, A. and Baldwin, I.T. (2003). Molecular technology reveals how the induced direct defenses of plants work. *Basic and Applied Ecology*, **4**, 15–26.
105. Roessingh, P., Städler, E., Fenwick, G.R., Lewis, J.A., Nielsen, J.K., Hurter, J., *et al*. (1992). Oviposition and tarsal chemoreceptors of the cabbage root fly are stimulated by glucosinolates and host plant extracts. *Entomologia Experimentalis et Applicata*, **65**, 267–82.
106. Rosenthal, G.A. and Berenbaum, M.R. (1991–1992). *Herbivores. Their interactions with secondary plant metabolites*, 2 Vols. Academic Press, San Diego.
107. Ruther, J. and Hilker, M. (1998). A versatile method for on-line analysis of volatile compounds from living samples. *Journal of Chemical Ecology*, **24**, 525–34.
108. Sakuma, M. (1998). Probit analysis of preference data. *Applied Entomology and Zoology*, **33**, 339–47.
109. Saxena, K. and Schoonhoven, L.M. (1978). Induction of orientational and feeding preferences in *Manduca sexta* larvae for an artificial diet containing citral. *Entomologia Experimentalis et Applicata*, **23**, 72–8.
110. Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C., *et al*. (2000). Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. *Proceedings of the National Academy of Sciences of the USA*, **97**, 11655–60.
111. Schoonhoven, L.M. (1967). Loss of hostplant specificity by *Manduca sexta* after rearing on an artificial diet. *Entomologia Experimentalis et Applicata*, **10**, 270–2.
112. Schoonhoven, L.M. and Derksen-Koppers, I. (1973). Effects of secondary plant substances on drinking behaviour in some Heteroptera. *Entomologia Experimentalis et Applicata*, **16**, 141–5.
113. Schoonhoven, L.M. and Derksen-Koppers, I. (1976). Effects of some allelochemics on food uptake and survival of a polyphagous aphid, *Myzus persicae*. *Entomologia Experimentalis et Applicata*, **19**, 52–6.
114. Schoonhoven, L.M. and Jermy, T. (1977). A behavioural and electrophysiological analysis of insect feeding deterrents. In *Crop protection agents. Their biological evaluation* (ed. N.R. McFarlane), pp. 133–46. Academic Press, London.
115. Simpson, S.J. (1995). Regulation of a meal: chewing insects. In *Regulatory mechanisms in insect feeding* (ed. R.F. Chapman and G. de Boer), pp. 137–56. Chapman & Hall, New York.
116. Schmelz, E.A., Engelberth, J., Alborn, H.T., O'Donnell, P., Sammons, M., Toshima, H., *et al.* (2003). Simultaneous analysis of phytohormones, phytotoxins, and volatile organic compounds in plants. *Proceedings of the National Academy of Sciences of the USA*, **100**, 10552–7.
117. Smith, J.J.B., Mitchell, B.K., Rolseth, B.M., Whitehead, A.T., and Albert, P.J. (1990). SAPID Tools: microcomputer programs for analysis of multi-unit nerve recordings. *Chemical Senses*, **15**, 253–70.
118. Smith, C.M., Khan, Z.R., and Pathak, M.D. (1994). *Techniques for evaluating insect resistance in crop plants*. CRC, Boca Raton.
119. Soule, S., Guntner, C., Vazquez, A., Argandona, V.H., Ferreira, F., and Moyna, P. (1999). Effect of *Solanum* glycosides on the aphid *Schizaphis graminum. Journal of Chemical Ecology*, **25**, 369–74.
120. Städler, E. (1977). Host selection and chemoreception in the carrot rust fly (*Psila rosae* F., Dipt. Psilidae): extraction and isolation of oviposition stimulants and their perception by the female. *Colloques Internationaux CNRS*, **265**, 357–72.
121. Städler, E. and Hanson, F.E. (1976). Influence of induction of host preference on chemoreception of *Manduca sexta*: behavioral and electrophysiological studies. *Symposia Biologica Hungarica*, **16**, 267–73.
122. Städler, E. and Roessingh, P. (1991). Perception of leaf surface chemicals by feeding and ovipositing insects. *Symposia Biologica Hungarica*, **39**, 71–86.
123. Stamp, N.E. and Bowers, M.D. (1994). Effects of cages, plant age and mechanical clipping on plant chemistry. *Oecologia*, **99**, 66–71.
124. Steinberg, S., Dicke, M., Vet, L.E.M., and Wanningen, R. (1992). Response of the braconid parasitoid *Cotesia (=Apanteles) glomerata* to volatile infochemicals: effects of bioassay set-up, parasitoid age and experience and barometric flux. *Entomologia Experimentalis et Applicata*, **63**, 163–75.
125. Thaler, J.S., Farag, M.A., Paré, P.W., and Dicke, M. (2002). Jasmonate-deficient plants have reduced direct and indirect defenses against herbivores. *Ecology Letters*, **5**, 764–74.
126. Thiéry, D. and Visser, J.H. (1986). Masking of host plant odour in the olfactory orientation of the Colorado potato beetle. *Entomologia Experimentalis et Applicata*, **41**, 165–72.
127. Tingey, W.M. (1986). Techniques for evaluating plant resistance to insects. In *Insect–plant interactions* (ed. J.R. Miller and T.A. Miller), pp. 251–84. Springer, New York.
128. Tinzaara, W., Dicke, M., Van Huis, A., Van Loon, J.J.A., and Gold, C.S. (2003). Different bioassays for investigating orientation responses of the banana

weevil, *Cosmopolites sordidus*, show additive effects of host plant volatiles and a synthetic male-produced aggregation pheromone. *Entomologia Experimentalis et Applicata*, **106**, 169–75.

129. Tjallingii, W.F. (1988). Electrical recording of stylet penetration activities. In *Aphids, their biology, natural enemies and control*, Vol. 2B (ed. A.K. Minks and P. Harrewijn), pp. 95–107. Elsevier, Amsterdam.
130. Tjallingii, W.F. (1995). Regulation of phloem sap feeding by aphids. In *Regulatory mechanisms in insect feeding* (ed. R.F. Chapman and G. de Boer), pp. 190–209. Chapman & Hall, New York.
131. Traynier, R.M.M. (1979). Long-term changes in the oviposition behaviour of the cabbage butterfly, *Pieris rapae*, induced by contact with plants. *Physiological Entomology*, **4**, 87–96.
132. Turlings, T.C.J., Davison, A.C., and Tamò, C. (2004). A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiological Entomology*, **29**, 45–55.
133. Van der Werf, W., Evans, E.W., and Powell, J. (2000). Measuring and modelling the dispersal of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in alfalfa fields. *European Journal of Entomology*, **97**, 487–93.
134. Van Helden, M. and Tjallingii, W.F. (2001). Experimental design and analysis in EPG experiments with emphasis on plant resistance research. In *Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior* (ed. G.P. Walker and E.A. Backus), pp. 144–72. Thomas Say Publications in Entomology, Proceedings of the Symposium at the XIX International Congress of Entomology, Beijing, China.
135. Van Poecke, R.M.P. and Dicke, M. (2003). Signal transduction downstream of salicylic and jasmonic acid in herbivory-induced parasitoid attraction by *Arabidopsis* is independent of JAR1 and NPR1. *Plant Cell and Environment*, **26**, 1541–8.
136. Vargas, C., Jadue, Y., and Araya, J.E. (1998). Technique for preserving fresh leaves for the study of minute arthropods, with reference to the false grape mite, *Brevipalpus chilensis. Zeitschrift für Pflanzenkrankheiten und Pflanzenzschutz*, **105**, 545–8.
137. Vet, L.E.M., van Lenteren, J.C., Heymans, M., and Meelis, E. (1983). An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. *Physiological Entomology*, **8**, 97–106.
138. Visser, J.H. (1976). The design of a low-speed wind tunnel as an instrument for the study of olfactory orientation in the Colorado potato beetle (*Leptinotarsa decemlineata*). *Entomologia Experimentalis et Applicata*, **20**, 275–88.
139. Visser, J.H., Piron, P.G.M., and Hardie, J. (1996). The aphids' peripheral perception of plant volatiles. *Entomologia Experimentalis et Applicata*, **80**, 35–8.
140. Voelckel, C. and Baldwin, I.T. (2003). Detecting herbivore-specific transcriptional responses in plants with multiple DDRT-PCR and subtractive library procedures. *Physiologia Plantarum*, **118**, 240–252.
141. Vogel, S. (1969). Low speed wind tunnels for biological investigations. In *Experiments in physiology and biochemistry* (ed. G.A. Kerkut), pp. 295–325. Academic Press, London.
142. Vrieling, K. and Derridj, S. (2003). Pyrrolizidine alkaloids in and on the leaf surface of *Senecio jacobaea* L. *Phytochemistry*, **64**, 1223–8.
143. Waage, J.K. (1983). Aggregation in field parasitoid populations: foraging time allocation by a population of *Diadegma* (Hymenoptera, Ichneumonidae). *Ecological Entomology*, **8**, 447–53.
144. Waller, D.A. and Jones, C.G. (1989). Measuring herbivory. *Ecological Entomology*, **14**, 479–81.
145. Waterman, P.G. and Mole, S. (1994). *Analysis of phenolic plant metabolites*. Blackwell. Oxford.
146. Wibe, A. (2004). How the choice of method influence on the results in electrophysiological studies of insect olfaction. *Journal of Insect Physiology*, **50**, 497–503.
147. Wiskerke, J.S.C. and Vet, L.E.M. (1994). Foraging for solitarily and gregariously feeding caterpillars: a comparison of two related parasitoid species. *Journal of Insect Behavior*, **7**, 585–603.
148. Wolfson, J.L. (1988). Bioassay techniques: an ecological perspective. *Journal of Chemical Ecology*, **14**, 1951–63.
149. Wolfson, J.L. and Murdock, L.L. (1987). Method for applying chemicals to leaf surfaces for bioassay with herbivorous insects. *Journal of Economic Entomology*, **80**, 1334–6.
150. Woodhead, S. (1983). Distribution of chemicals in glass fibre discs used in insect bioassays. *Entomologia Experimentalis et Applicata*, **34**, 119–20.
151. Xia, Q.Y., Zhou, Z.Y., Lu, C., Cheng, D.J., Dai, F.Y., Li, B., *et al*. (2004). A draft sequence for the genome of the domesticated silkworm (*Bombyx mori*). *Science*, **306**, 1937–40.
152. Zhang, A., Linn, C., Wright, S., Prokopy, R., Reissig, W., and Roelofs, W. (1999). Identification of a new blend of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella*. *Journal of Chemical Ecology*, **25**, 1221–32.

Taxonomic Index

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