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]

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

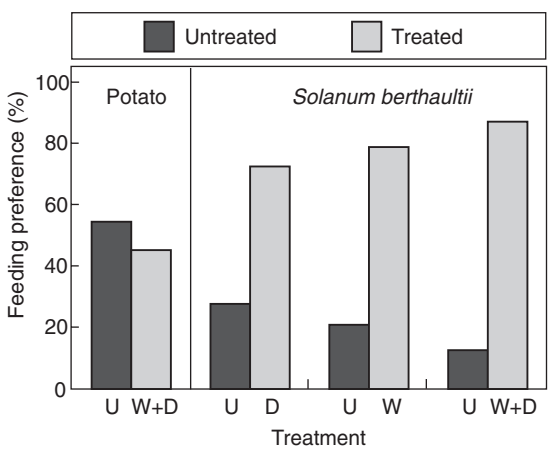

Figure 7.1 Effect of trichome removal of susceptible potato and resistant *Solanum berthaultii* by dipping (D, 95% ethanol dip), wiping (W, soft bristle-brush wipe), or combined dipping and wiping (W + D). Preference for treated versus untreated (U) leaves in adult Colorado potato beetles was determined 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]

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

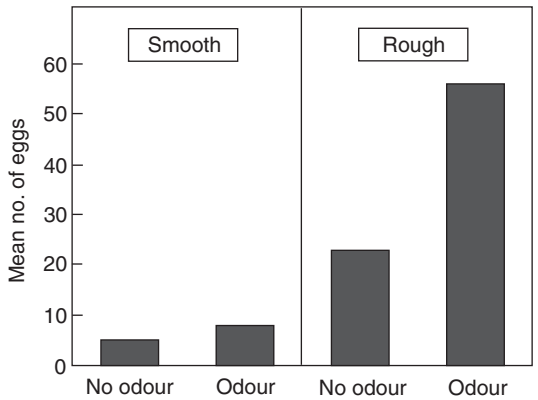

Figure 7.2 Effects of combinations of mechanosensory and olfactory cues on oviposition by the diamondback moth *Plutella xylostella*. Smooth or rough plastic caps were offered as an oviposition substrate, with or without 10 ppm allylisothiocyanate as the odour (this compound is a major volatile released 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]

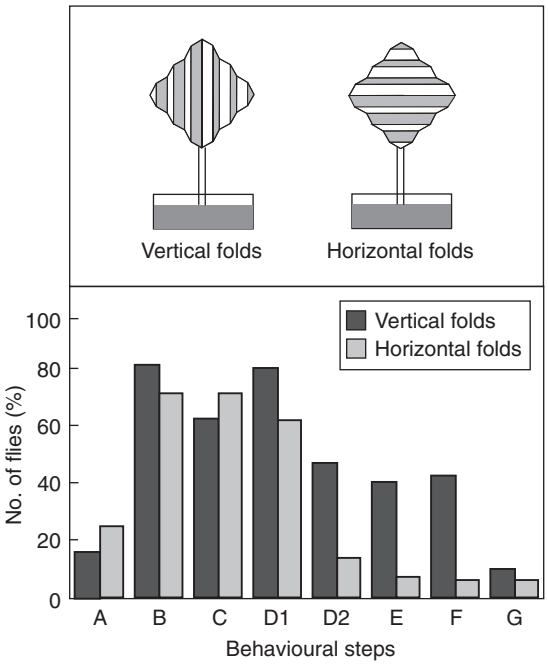

Figure 7.3 Influence of mechanosensory quality (horizontal versus vertical folds) of paper model leaves on oviposition behaviour of the cabbage root fly *Delia radicum*. For each behavioural element (A–G), the percentage of flies performing this step is displayed. A, short visit, no exploration of leaf; 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]

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]

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,

* 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 (see Table 4.7). 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.

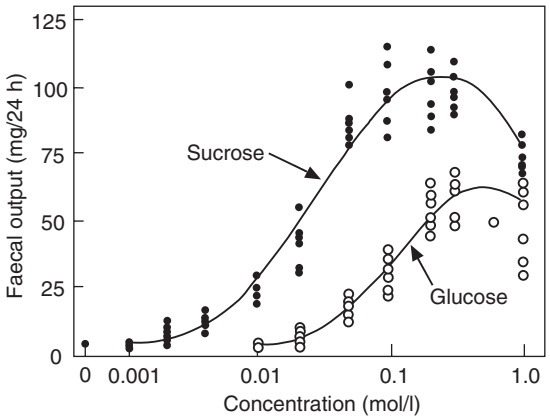

Figure 7.4 Behavioural response of *Pieris brassicae* larvae to two sugars, sucrose and glucose, incorporated in an agar-based gel medium (a mixture of agar, water, and cellulose). The parameter on the ordinate is dry weight of faecal output produced by six larvae over 24 h, a fair indicator of the amount 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 europaea*	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.	*Brassic*a 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	145	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		

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]

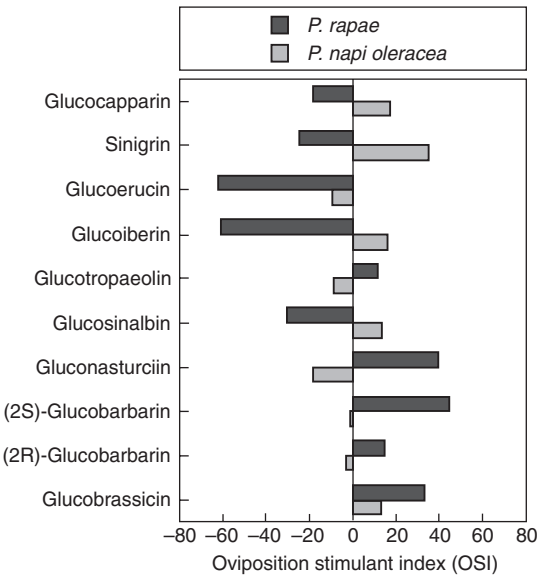

Figure 7.5 Stimulation of oviposition in *Pieris rapae* and *P. napi oleracea* by pure glucosinolates when sprayed on the non-host Lima bean (2 ml of a 0.1 mmol/l solution in water). The oviposition stimulant index (OSI) signifies the degree of preference in a dual-choice situation relative to a Lima 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]

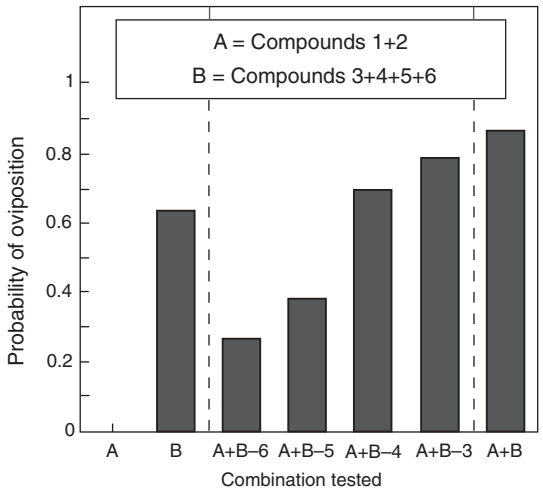

Figure 7.6 Probability of oviposition by individual females of *Papilio protenor* on filter-paper discs treated with different combinations of compounds isolated from the host plant *Citrus unshui*. Compounds tested were: (1) naringin 0.1%, (2) hesperidin 0.05%, (3) proline 0.2%, (4) synephrine 0.1%, (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]

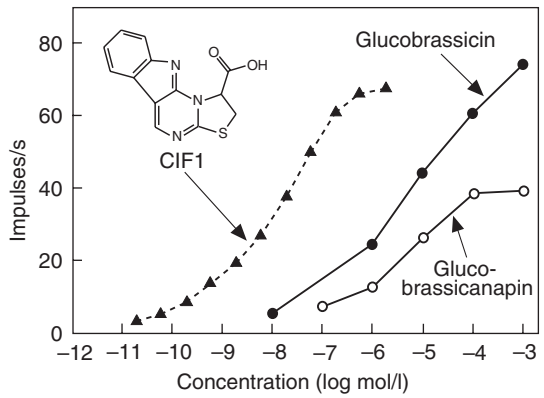

Figure 7.7 Dose–response curves of neural activity (number of action potentials in the first second after contact with sensillar tip) in taste hairs on the fifth tarsomere of cabbage root flies (*Delia radicum*) for the glucosinolates glucobrassicin and glucobrassicanapin, the strongest glucosinolate 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
	Chlorogenic acid	*Solanum tuberosum* (Solanaceae)	*Leptinotarsa decemlineata* (Coleoptera)	88

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	$\leq$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

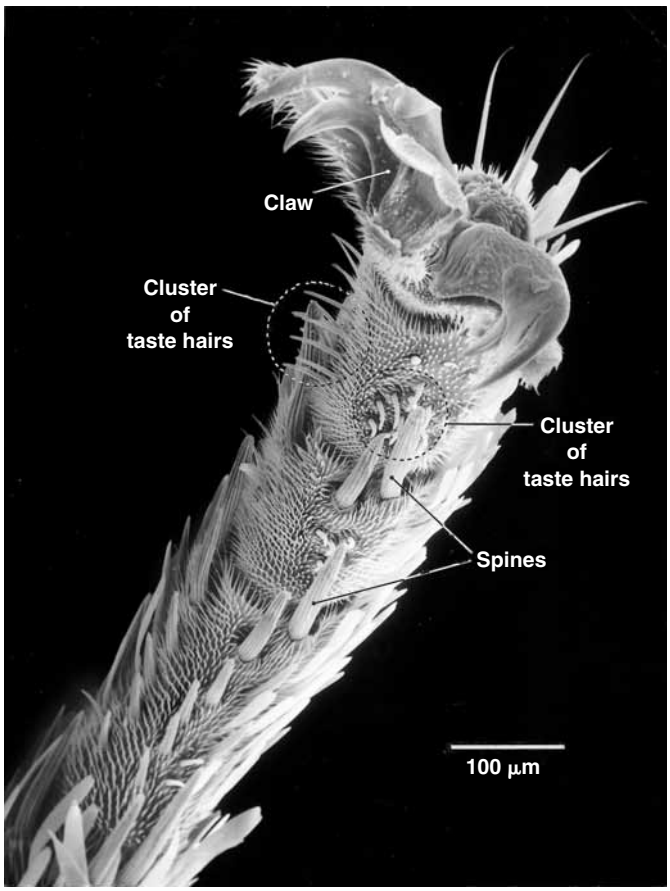

Figure 7.8 Scanning electron micrograph of the ventral side of the two distal tarsomeres of the prothoracic leg of a female *Pieris rapae* butterfly. Clusters of chemosensory hairs occur close to larger, non-innervated spines. (Reproduced by courtesy of E. Städler, Wädenswil, Switzerland).

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.[31] 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

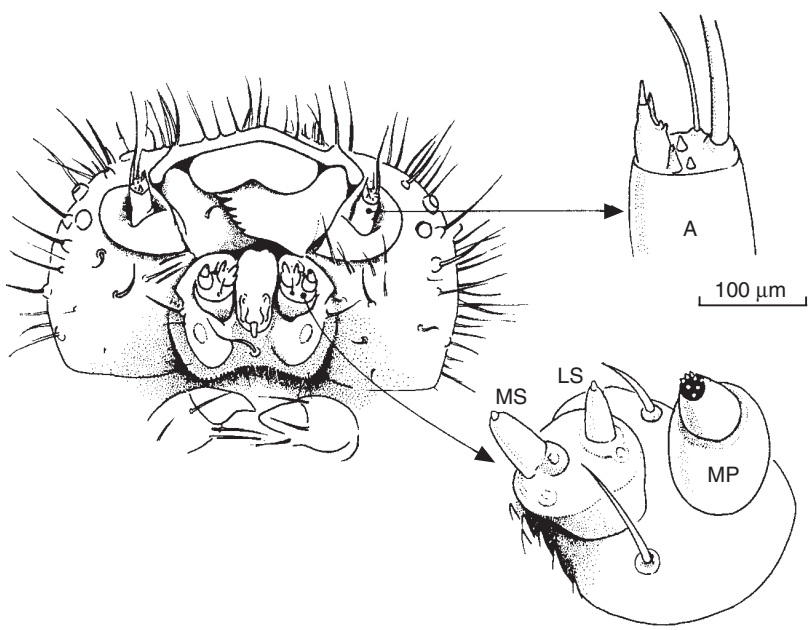

Figure 7.9 Diagram of the head of a caterpillar seen from below with enlargements of an antenna (A) and a maxilla. MP, maxillary palp; LS and MS, lateral and medial sensilla styloconica.

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 orrelates 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]

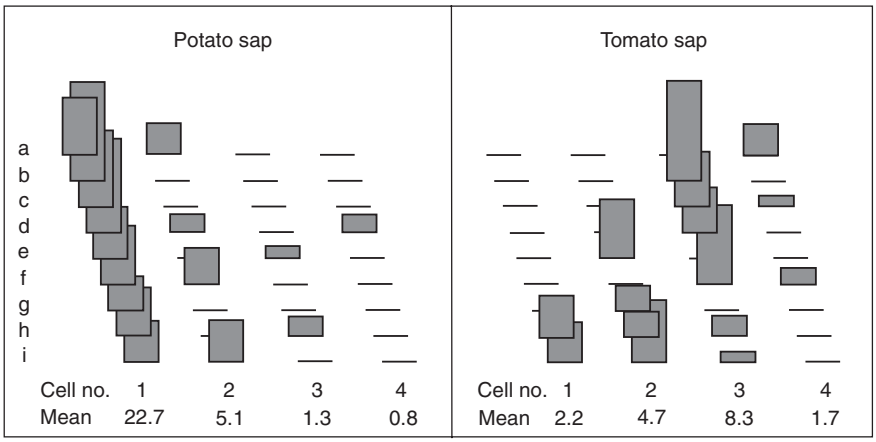

Figure 7.10 Across-fibre patterns of nine individual Colorado potato beetles (a–i) in response to leaf saps of potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*). The activity levels of four cells in taste sensilla on the galea of adults are represented as bars (mean values over nine 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]

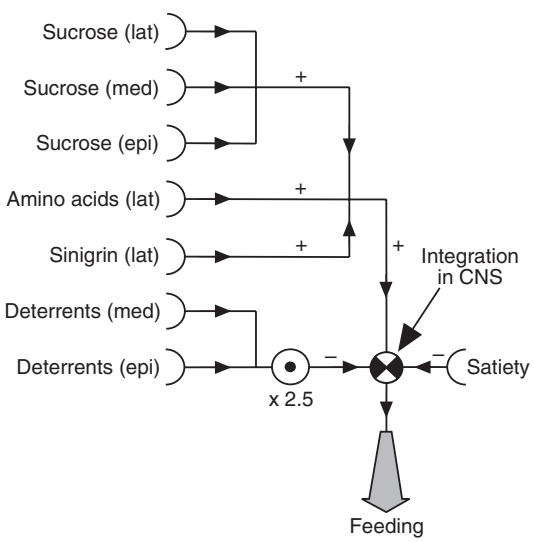

Figure 7.11 Schematic representation of how the inputs from different mouthpart chemoreceptors might be integrated within the central nervous system (CNS) to regulate feeding in the caterpillar of *Pieris brassicae*. Impulses from the sucrose, amino acid, and glucosinolate cells in the lateral (lat) 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/medical 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 amaricana.

* 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]

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

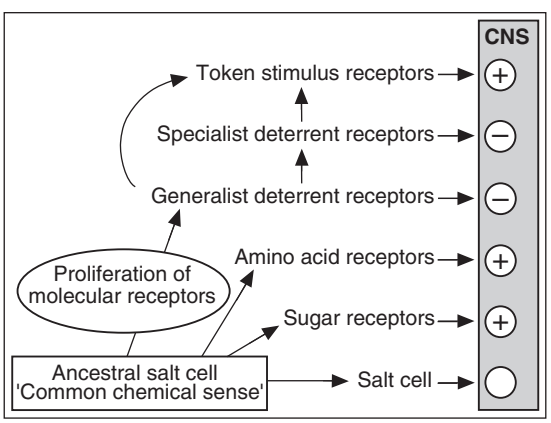

Figure 7.12 Hypothetical evolutionary pathways of gustatory receptor types in specialist herbivores. The circles containing plus and minus signs depicted in the central nervous system (CNS) represent excitatory and inhibitory synapses with the first-order interneurons. (From Schoonhoven and van Loon, 2002.)[190]

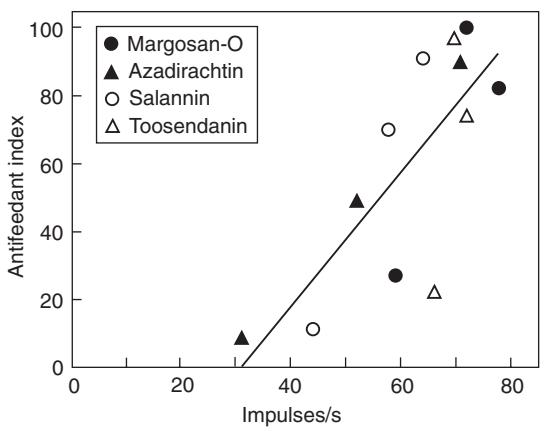

Figure 7.13 Relationship between antifeedant index (as determined by dual-choice tests) and spike frequencies of a deterrent receptor cell in the medial sensillum styloconicum of *Pieris brassicae* larvae. Impulse frequencies in response to three different concentrations of Margosan-O®, azadirachtin, 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

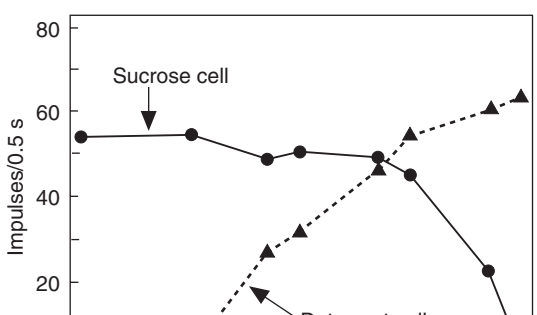

Figure 7.14 Impulse frequencies of the sucrose-sensitive and deterrent cells in the lateral sensillum styloconicum of *Heliothis subflexa* larvae upon stimulation with 5 mmol/l sucrose mixed with different concentrations of sinigrin. (Modified from Bernays and Chapman, 2000.)[12]

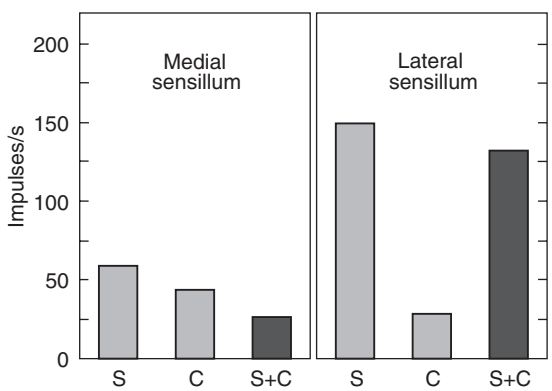

Figure 7.15 Inhibitory effects of cyanin chloride, an anthocyanin, on sugar responses in the two maxillary sensilla styloconica of *Pieris brassicae* larvae. Responses are presented as total impulse frequencies when stimulated with 15 mmol/l sucrose (S), 2.5 mmol/l cyanin chloride (C), and a mixture 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]

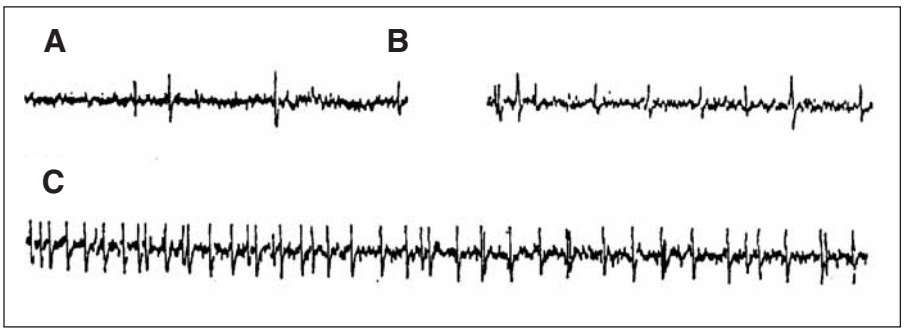

Figure 7.16 Synergistic receptor responses in the medial sensillum styloconicum on the maxilla of *Isia isabella* larvae. (**A**) Response to 0.001 mol/l sinigrin. (**B**) Response to 0.1 mol/l sucrose. (**C**) Response to a mixture of sinigrin and sucrose. The cell that responds preferentially to sinigrin 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

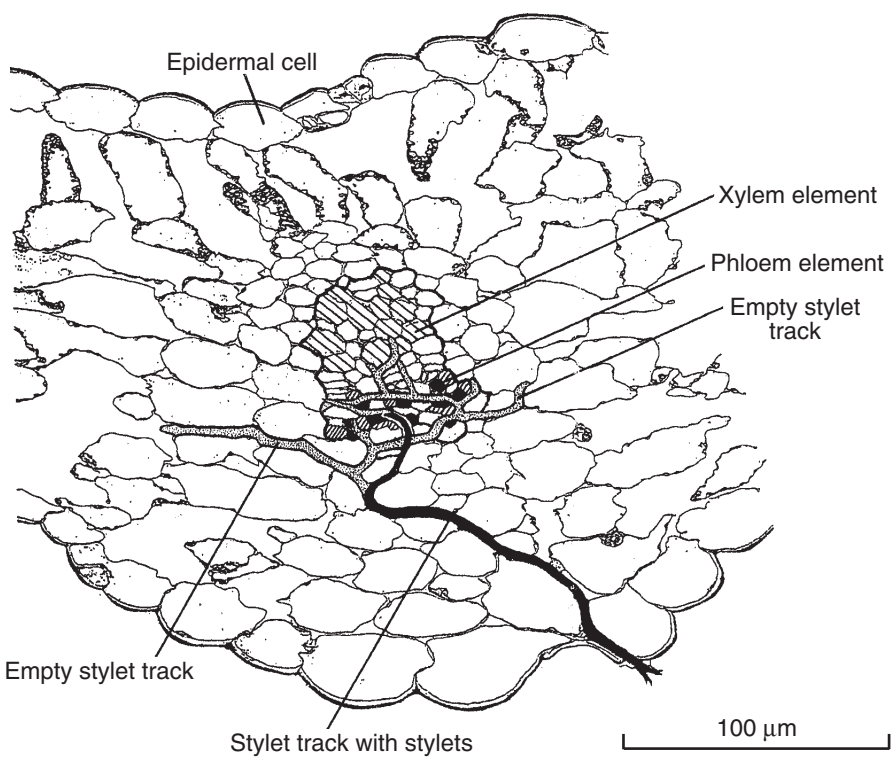

Figure 7.17 Stylet pathway of an aphid (*Aphis fabae*) feeding on a sieve element in the vein of a broad bean leaf. The stylet track shows many branches, representing earlier search movements during the process of phloem localization. The empty branches consist of salivary sheath material, which remains 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, 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.

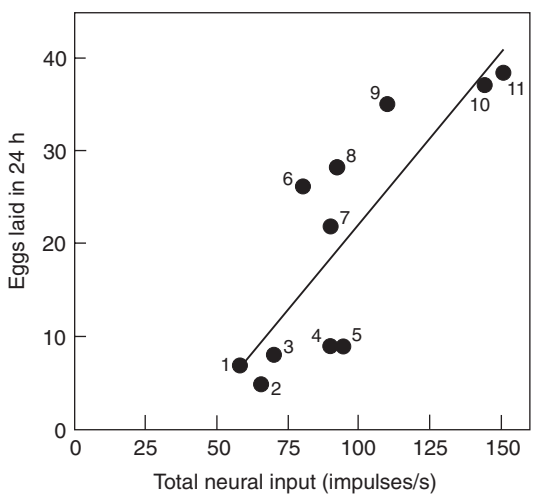

Figure 7.18 Relationship between summed neural input (impulses in the first second of stimulation) from two different receptor types on the legs and from labellar sensilla in the turnip root fly *Delia floralis* and oviposition behaviour (number of eggs laid over a 24-h period in a no-choice situation) 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

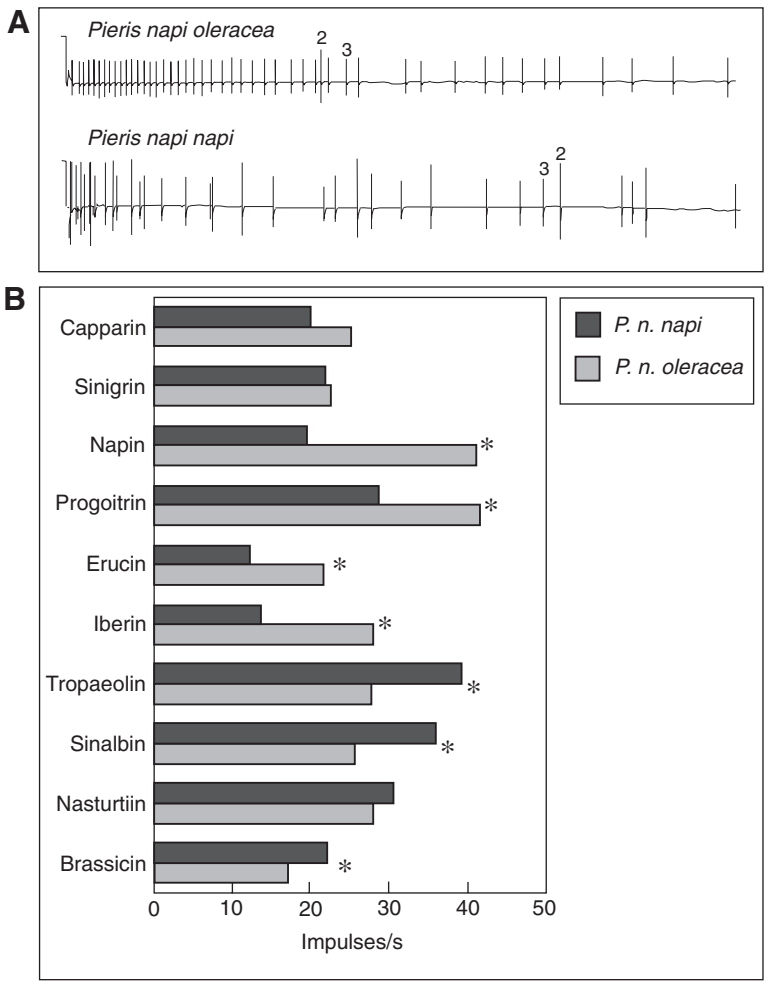

Figure 7.19 (**A**) Recordings of electrophysiological activity from taste hairs on tarsi of female *Pieris napi oleracea* and *P. napi napi* in reponse to the glucosinolate gluconapin at 10 μg/ml. In *P. napi napi* a second cell (designated as '2') fires much more frequently than in *P. napi oleracea*. (**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]

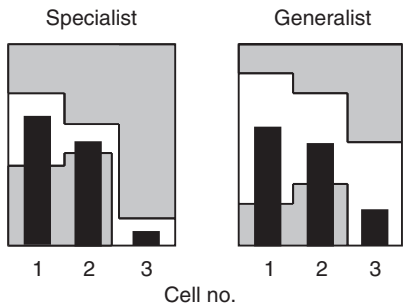

Figure 7.20 Model of CNS processing of sensory input in a food specialist and a generalist. The black bars represent action potential frequencies in three chemoreceptors (1–3) when stimulated by an acceptable food plant. The white space of the 'lock' reflects the variation permitted to the sensory input while still being interpreted as acceptable. Cell 3 is a deterrent receptor. (From Schoonhoven, 1987.)[182]

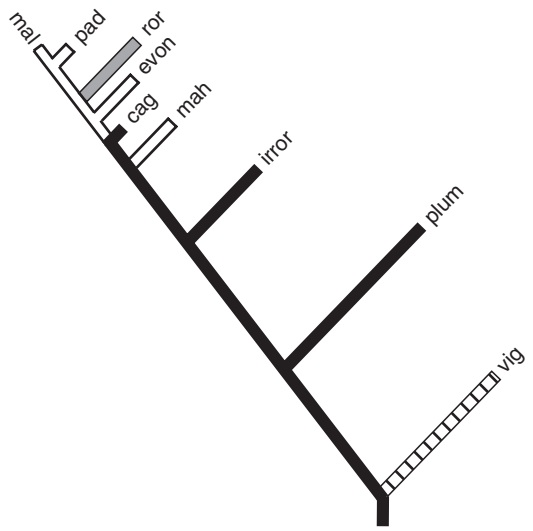

Figure 7.21 Phylogenetic tree of the nine west European *Yponomeuta* species based on allozyme data and the botanical status of their host plants. *Yponomeuta* species: cag, *cagnagellus*; evon, *evonymellus*; irror, *irrorellus*; mah, *mahalebellus*; mal, *malinellus*; pad, *padellus*; plum, *plumbellus*; 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.[140] 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]

Evolutionary changes in host-plant specialization, becoming either more or less host specific, switches to closely or to distantly related new host-plant species and rapid changes in genetically based host-plant preferences which might occur in only seven generations,[198] depend on various kinds of genetic and developmental constraints on mutational changes in the insect's genome, which are assumed to occur stochastically. 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.

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. (11993). 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.

AQ: Please check, we have deleted the reference 59 it was repeated twice. Please check is it okay.

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.
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**, 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*,

AQ: Please check reference 162 for complete details.

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. *BioScienc*e, **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. (1995c). 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.J.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.

AQ: Please check Shield, V.D.C 1995b is not provided.

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.