

Figure 4.17. Responses of *Locusta migratoria* on successive encounters with the nonhost, *Senecio vulgaris*. Initially, most insects bit the leaf before rejecting it. With each successive encounter, a greater percentage rejected following palpation without biting (after Blaney and Simmonds, 1985).

4.2.4 The internal constituents of the leaf

When an insect bites into a leaf it releases the cell contents and, we believe, these flow over the mouthparts and stimulate the contact chemoreceptors. In grasshoppers, tracts of noninnervated hairs on the innerside of the mouthparts appear to direct the plant fluids to the receptors (Fig. 4.18). These hairs are easily wetted by water while the rest of the cuticle resists wetting. Functionally similar adaptations probably exist in other insects although they have not been investigated.

The plant cell contains large numbers of different chemicals, many of which have the capacity to stimulate the contact chemoreceptors on the mouthparts. Some will be phagostimulatory and others deterrent. It is convenient and necessary to consider them separately, but we emphasize again that the insect response depends on the overall sensory input and may not reflect the sum of the responses to the individual components.

4.2.4.1 Phagostimulants

The principal phagostimulants are nutrients, and especially sugars. In general, the same sugars are stimulating for different species (Table 4.5), sucrose and fructose generally being the most effective. Pentose sugars are not usually stimu-

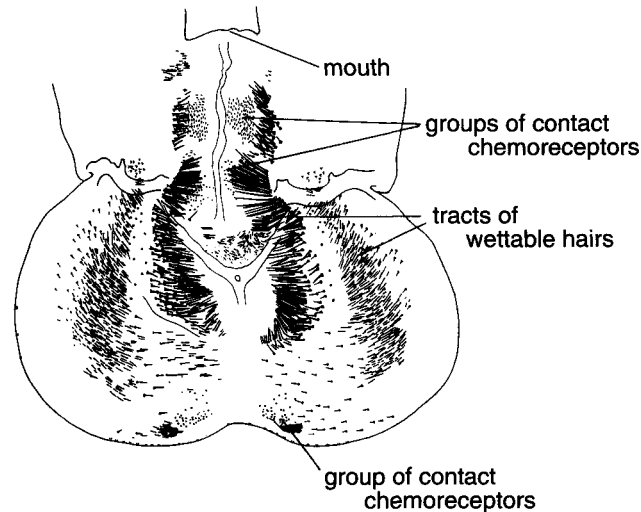


Figure 4.18. Inside of the labrum of a grasshopper, showing the positions of tracts of wettable hairs. It is probable that these tracts conduct plant sap to the groups of contact chemoreceptors just outside the mouth (after Chapman and Thomas, 1978).

lating. In all cases, the effectiveness of the sugar, as measured by the meal size or the amount of an artificial substrate consumed over a period, increases with its concentration (Fig. 4.19a) at least within the limits occurring in most plants.

Despite the importance of proteins nutritionally, there is, as yet, no good evidence that insects can taste protein. They can, however, usually taste some amino acids (Table 4.5), although the stimulating power of these compounds is usually low compared with sugars (Fig. 4.19a). Consequently, for most insects feeding on most plants, phagostimulatory effects are likely to be dominated by sugars. In a caterpillar and a grasshopper, it has been shown that a sucrose concentration equal to that occurring in host plants is sufficient to make the insect eat a maximum-sized meal on an otherwise neutral substrate. In addition, it has been shown that the amount of feeding on leaves is correlated with their sugar content (Fig. 4.20). Such examples do not prove that sugar is the only factor affecting feeding, but they indicate that the sugar content of leaves is likely to be very important in food selection. However, amino acids may affect meal duration or the intervals between meals, and insects are able to distinguish artificial diets that are high in protein from those that are not by associative learning (Section 6.4).

Phospholipids and some nucleotides may also be phagostimulatory (Table 4.5), although the latter are much less important for phytophagous insects than for blood-sucking insects. Inorganic salts, which are essential nutrients, usually

Table 4.5. Phagostimulatory effect of some nutrient compounds tested singly for some phytophagous insects. + = weakly stimulating, +++++ = strongly stimulating, — = no effects, • = not tested.

Compounds	<i>Locusta migratoria</i> (Orthoptera)	<i>Aphis fabae</i> (Hemiptera)	<i>Oncopeltus fasciatus</i> (Hemiptera)	<i>Leptinotarsa decemlineata</i> (Coleoptera)	<i>Pieris brassicae</i> (Lepidoptera)
Carbohydrates					
Pentoses					
D-ribose	—	•	•	—	—
D-xylose	—	•	•	—	—
Hexoses					
D-fructose	+++++	•	•	+	—
D-glucose	+++	+	•	+	++
L-sorbose	+	•	•	—	—
Disaccharides					
D-cellobiose	—	•	•	—	—
D-maltose	+++++	+	•	—	—
D-sucrose	+++++	+++++	•	+++++	+++++
Sugar Alcohols					
Inositol	+	•	•	—	—
Sorbitol	+	—	•	—	—
Amino Acids					
L-alanine	•	++	++	++	•
γ-aminobutyric acid	—	•	+	++	—
L-methionine	—	++	++	—	—
L-serine	+	++	+	++	—
L-phenylalanine	—	—	+	—	—
L-tyrosine	—	—	•	—	•
L-proline	+	++	•	++	+
Nucleotides					
AMP	—	•	•	—	•
ADP	—	•	•	—	•
ATP	—	•	•	—	•

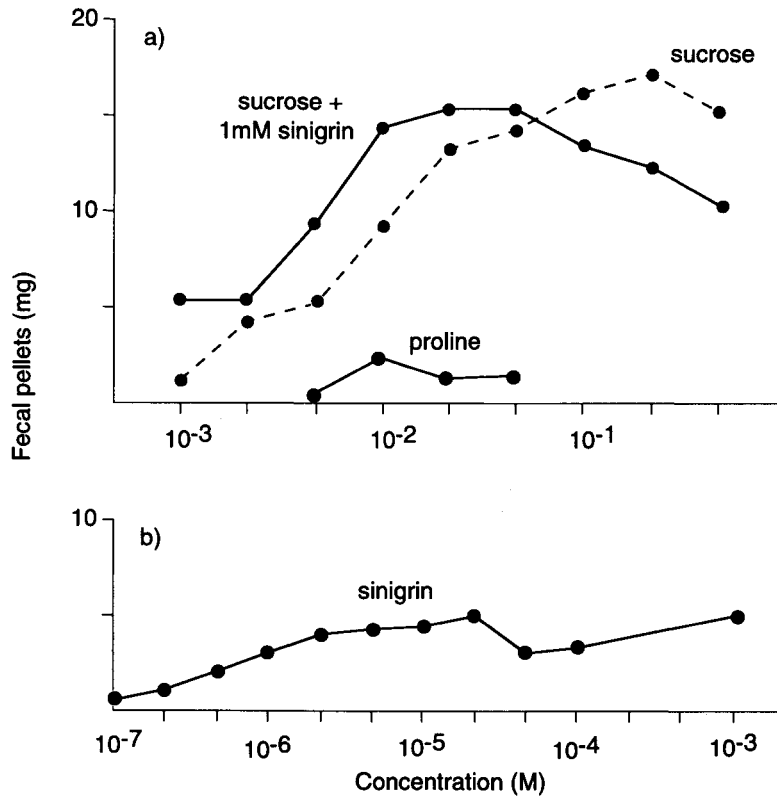


Figure 4.19. Responses of caterpillars of *Pieris brassicae* to some phagostimulants. The amount eaten was determined by measuring the dry weight of fecal pellets produced in a 24-hour period. Insects were fed on blocks of agar/cellulose to which the test chemical was added (after Blom, 1978).

have no effect at the concentrations at which they occur in plants. At higher concentrations they become deterrent.

These various compounds will be present in all plants and will contribute to a plant's acceptability. But they cannot provide the information necessary to confer specificity and no examples are known of host-plant specificity based on the presence of a particular nutrient or a particular combination of nutrients.

There are also many examples of plant secondary compounds acting as phagostimulants. In some cases these are widely-occurring compounds that affect insects feeding on a range of different plants. The flavonoid glycoside rutin is an example. It occurs in many different plant families and stimulates feeding in polyphagous species, like the larva of *Helicoverpa zea* and the grasshopper, *Schistocerca americana*. Other examples are listed in Table 4.6. It is often true

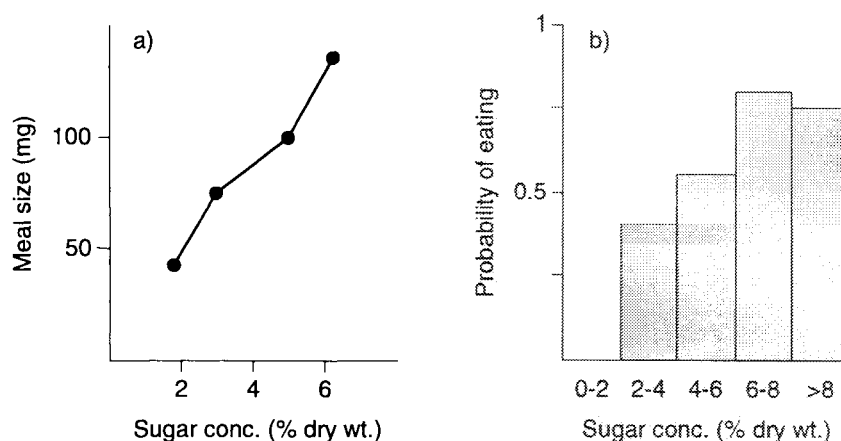


Figure 4.20. Effects of sugar concentration in plants on feeding. **a)** Meal sizes taken by fifth instar nymphs of *Schistocerca gregaria* on seedling wheat (EAB, unpublished). **b)** The probability that larvae of *Tyria jacobaeae*, the cinnabar moth, would feed on leaves with different sugar levels. There was no difference in the probability of feeding in relation to protein levels in the same leaves (after Soldaat, 1991.)

that these phagostimulatory effects are only observed when the compounds are present in low concentrations.

In a many other cases, however, particular secondary compounds are only found in one or a small number of plant taxa. In these cases, the chemicals can provide indicators or sign stimuli to a monophagous or oligophagous insect that it is on the correct host and so help to define its host range. For example, the caterpillars of the butterfly subfamily Pierinae feed almost exclusively on cruciferous plants. These are characterized by glucosinolates which have been found to be phagostimulatory for larvae and oviposition stimulants for females of Pierinae as well as for many other insects that are oligophagous on these plants. Iridoid glycosides, which are monoterpenoids, characterize the host plants of the buckeye butterfly, *Junonia coenia*, and the checkerspot butterflies, *Euphydryas* spp., and are phagostimulants and oviposition stimulants for them. There are many other examples of insect genera or species in which phagostimulatory effects are produced by chemicals that are characteristic of the host plant (Table 4.7).

Sometimes the chemicals are phagostimulatory by themselves. This is true of some glucosinolates for crucifer-feeding insects and of populin for the beetle, *Chrysomela vigintipunctata*. In other cases, the chemical may have no effect by itself, but may synergise feeding on an artificial diet containing sugar. Fig. 4.21 shows an example of this for sinigrin and the diamondback moth, *Plutella xylostella*. It is also often true that the addition of an appropriate sign stimulus chemical to a leaf of an unacceptable plant will cause an insect tuned to this substance to eat the treated leaf.

Table 4.6. Some plant secondary compounds that are produced by plants in many different families and which are phagostimulatory for some insects. In some cases phagostimulation only occurs when the chemical is present in low concentrations.

Chemical	Chemical class	Insects that are phagostimulated
Anthraquinone	quinone	<i>Schistocerca gregaria</i> (grasshopper)
Caffeic acid	phenolic acid	<i>Bombyx mori</i> (caterpillar)
Chlorogenic acid	phenylpropanoid acid	<i>Leptinotarsa decemlineata</i> (beetle); <i>Bombyx mori</i> (caterpillar)
Chrysophanol	quinone	<i>Schistocerca gregaria</i> (grasshopper)
Cinnamic acid	phenylpropanoid	<i>Schistocerca gregaria</i> (grasshopper)
Quercitrin	flavonoid glycoside	<i>Anthonomus grandis</i> (beetle); <i>Bombyx mori</i> (caterpillar)
Linamarin	cyanogenic glycoside	<i>Epilachna varivestis</i> (beetle)
Luteolin-7-glucoside	flavonoid glycoside	<i>Chrysomela vigintipunctata</i> (beetle)
Rutin	flavonoid glycoside	<i>Schistocerca americana</i> (grasshopper); <i>Plagioderia versicolora</i> (beetle); <i>Heliothis virescens</i> (caterpillar)
Tannic acid	tannin	<i>Anacridium melanorhodon</i> (grasshopper); <i>Lymantria dispar</i> (caterpillar)

Table 4.7. Some plant secondary compounds that are taxon specific and phagostimulatory for some insects. It is believed that these compounds have a major role in defining the host ranges of the insects named.

Chemical	Chemical class	Plant taxon	Insects that are phagostimulated
Catalpol	monoterpenoid	<i>Catalpa</i>	<i>Ceratonia catalpae</i> (caterpillar)
Catalpol	monoterpenoid	plantains	<i>Euphydryas chalcedona</i> (caterpillar)
Cytisine	alkaloid	broom	<i>Uresiphita reversalis</i> (caterpillar)
Gossypol	sesquiterpene	cotton	<i>Anthonomus grandis</i> (beetle)
Hypericin	quinone	St. John's wort	<i>Chrysolina brunsvicensis</i> (beetle)
Monocrotaline	alkaloid	<i>Crotalaria</i>	<i>Utetheisa ornatrix</i> (caterpillar)
Morin	flavonoid	mulberry	<i>Bombyx mori</i> (caterpillar)
Nordihydroguaiaretic acid	phenylpropanoid	creosote bush	<i>Boottettix argentatus</i> (grasshopper)
Phloridzin	flavonoid	apple	<i>Aphis pomi</i> (aphid)
Populin	phenolic	willow	<i>Chrysomela vigintipunctata</i> (beetle)
Salicin	phenolic	willow	<i>Plagioderia versicolora</i> (beetle); <i>Laothoe populi</i> (caterpillar)
Sinigrin	glucosinolate	cabbage family	<i>Brevicoryne brassicae</i> (aphid); <i>Phyllotreta</i> (beetle); <i>Pieris brassicae</i> (caterpillar); <i>Athalia proxima</i> (sawfly larva)
Sparteine	alkaloid	broom	<i>Acyrtosiphon spartii</i> (aphid)

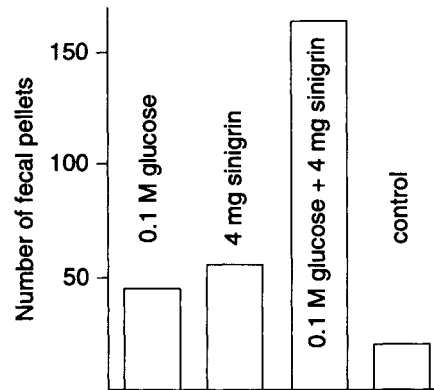


Figure 4.21. The synergistic effect of sinigrin with glucose. Larvae of *Plutella xylostella* were fed on cellulose-agar gels to which glucose, sinigrin or both were added. Glucose or sinigrin alone had slight phagostimulatory effects compared with the control (cellulose-agar alone). A mixture of the two in the same concentrations greatly enhanced the amount eaten over an 18-hour period. Amount eaten is expressed as the number of fecal pellets produced by 10 larvae (after Nayar and Thorsteinson, 1963).

These data leave no doubt that such chemicals have a special significance to the insects and play important roles in host-plant recognition. Probably, in a majority of instances, they provide a principal basis for monophagy and oligophagy. However, precisely how they function in host-plant selection is not clear. For most phytophagous species examined, sugars are major phagostimulants and the insects will often eat sugar-based diets even in the absence of the host-identifying chemical. For example, in *Pieris brassicae* glucosinolates clearly have a phagostimulatory effect. However, the effect of sinigrin on food intake over a 24 hour period is small compared with the effects of sucrose alone (Fig. 4.19). Sucrose at concentrations above 5×10^{-2} M, approximately that occurring in the plant, maximizes food intake; the insect cannot eat any more so that the addition of sinigrin can have no effect. It only has an effect on food intake when the concentration of sucrose is low. When the effects of sinigrin on first meal length and first feeding bout are considered, instead of food intake over 24 hours, it is found to have no effect when mixed with 3×10^{-1} M sucrose, but some effect was observed with 3×10^{-3} M sucrose. The sugar level is probably never as low as this in the plant. Most insect/plant relations have not been investigated with the thoroughness of the *Pieris/Brassica* association, and there is no case in which we have a clear idea of how the supposed sign stimulus has its effect.

However, it may be true that in whole plants the effects of host-specific chemicals are synergised by other constituents that enhance their phagostimulatory effect well above that of the nutrient phagostimulants. This thesis has been argued in particular in the case of a number of beetles feeding on Brassicaceae. For example, sinigrin alone is not a phagostimulant for the weevil, *Ceutorhynchus constrictus*, but it is highly effective when presented together with other chemicals from the host plant which are believed to be flavonoids. Perhaps the combined phagostimulatory effect is greater than that of the nutrients in the plant, although this has not been investigated. The possible importance of combinations of chemicals is also suggested by the increasing number of studies on oviposition behavior that demonstrate synergistic effects between a number of different compounds (Section 4.2.3). Comparably detailed studies on phagostimulants have not generally been undertaken.

These effects could arise through the interaction of chemicals at the insect's sensory receptors (see Section 3.2.3) so that the sign stimulus dominates the information that the insect receives. So far only one study in which the activities of different neurons have been distinguished has investigated the neurophysiological responses of oligophagous insects to a range of saps from host and nonhost plants. One of the species examined in this study was the Colorado potato beetle, *Leptinotarsa decemlineata*, which is oligophagous on a number of solanaceous plants. No particular group of chemicals is known to provide sign stimuli for this species, but the results of the study are important in the current context.

Four species of Solanaceae were examined: *Solanum tuberosum* (potato) and *Solanum dulcamara* (nightshade), which were readily accepted as food, and *Solanum elaeagnifolium* (horse nettle) and *Lycopersicon esculentum* (tomato), which were less acceptable. Stimulation with the sap of *S. tuberosum* usually produced a response in several different cells. One of these (cell 1 in Fig. 4.22a) fired whenever the stimulus was applied; the other cells were much less consistent. In addition, the firing rate of cell 1 was much higher than that of any of the other cells (Fig. 4.22c). A similar pattern was observed when *S. dulcamara* was the stimulus, but with the other two plants the pattern was different. With *L. esculentum*, none of the cells fired consistently (Fig. 4.22b) and none fired at a high rate. With *S. elaeagnifolium*, the pattern was different again. Cell 1 fired consistently at a high rate, much as it did with the more acceptable plants, but cell 2 was also active and fired at a relatively high rate.

The pattern of activity that distinguishes highly acceptable from less acceptable plants is the high and consistent firing rate of cell 1 in the absence of significant activity of other cells. It has been suggested that, in addition, the high variability of the responses of the other cells is itself used as a signal. The insect regards a high level of variability in a sequence of stimulations as "foreign," or not acceptable.

This type of interpretation did not fit as well to the behavior of two other species of *Leptinotarsa* examined, but these results are based on an investigation of only one sensillum. If other sensilla on the mouthparts had been examined,

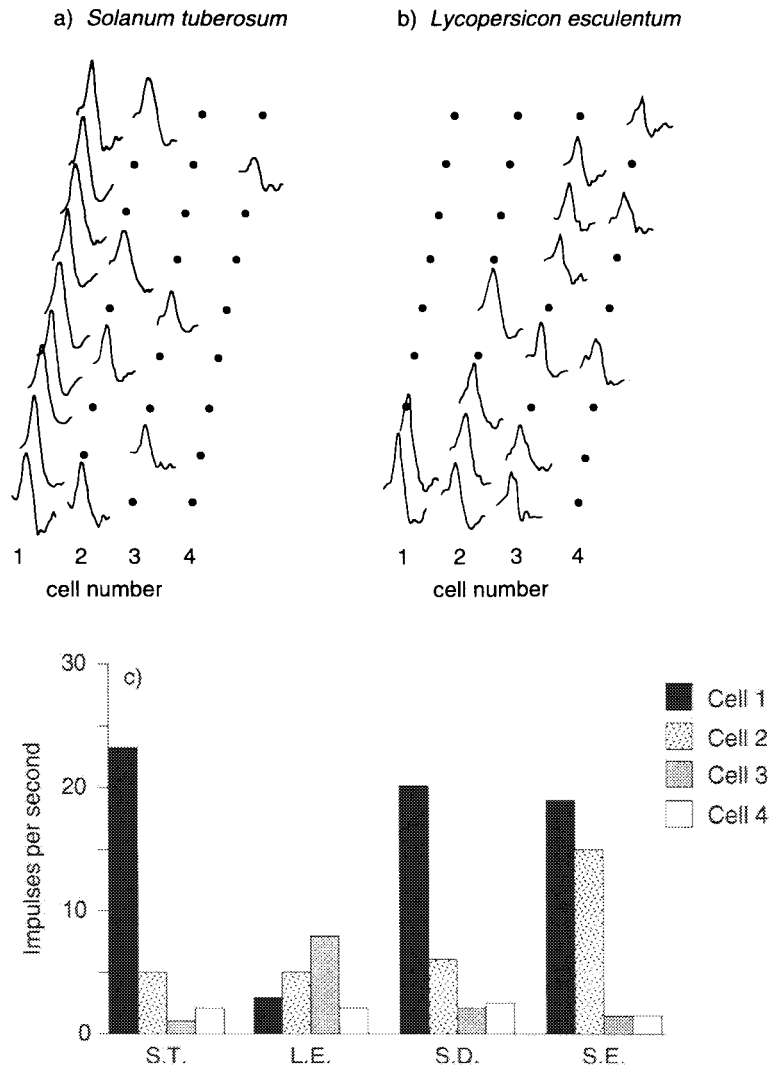


Figure 4.22. Responses of sensory neurons in the galeal sensillum of adult *Leptinotarsa* to the sap of **a**) potato, which is eaten readily, and **b**) tomato, which is less readily eaten. The different neurons within the sensillum are categorized by the shapes of the action potentials. Each horizontal line represents a single stimulation (there were nine stimulations for each plant sap). Cell one always fired when the sensillum was stimulated with potato sap. No other cell responded regularly to either of the saps. **c**) The average number of action potentials produced in the first second of stimulation by saps of acceptable (*Solanum tuberosum* (S.T.) and *S. dulcamara* (S.D.)) and less acceptable (*Lycopersicon esculentum* (L.E.) and *S. elaeagnifolium* (S.E.)) plants (after Sperling and Mitchell, 1991).