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Insect behavior at the leaf surface and learning as aspects of host plant selection

R. F. Chapman and E. A. Bernays

Department of Entomological Sciences and Division of Biological Control, University of California, Berkeley (California 94720, USA)

Summary. Direct observations on the feeding behavior of insect herbivores are uncommon, but important. The important aspects of host-plant selection by phytophagous insects that have been revealed by such observations are the role of chemicals in the leaf surface, and learning. There are few detailed reports of behavior at the leaf surface, but these indicate that many, if not all, insects exhibit behavior patterns that can be interpreted as an examination of the quality of the surface and acceptance or rejection may follow without further testing. A number of experiments show that chemicals from the leaf surface commonly contribute to the acceptability or otherwise of a plant and in most cases so far the active chemicals are of widespread occurrence, not having a specific association with the host plant. Some experiments show that the association between surface chemicals and plant palatability is learned, but in other cases there is evidence of an innate response. Habituation to deterrent chemicals has been demonstrated in the laboratory, but not in the field. Food aversion learning also occurs and may be important in dietary switching by polyphagous insects.

Key words. Behavior; learning; leaf surface; wax, insects; food selection; secondary compounds.

It is generally accepted that the food selection behavior of phytophagous insects is largely governed by the distribution of secondary chemicals in plants. Much of the evidence for this is based on experiments that record the outcome of an insect's behavior, commonly in terms of the amount eaten, but only rarely is the behavior itself described. This is probably unimportant in the development of certain evolutionary concepts, but for a proper understanding of the role of chemicals in insect ecology a fuller knowledge of the behavior is necessary. One reason for this is that the instantaneous behavior of an insect that leads it to accept or reject a particular plant may not be reflected in observations of the amount eaten after an interval. For example, on the basis of long-term feeding studies, it has become generally accepted that the range of host plants within the Solanaceae eaten by the Colorado potato beetle, *Leptinotarsa decemlineata*, is governed by the distribution of alkaloids. The tomato glycoalkaloid tomatine is one that has a deterrent effect[29]. However, infusing tomatine into potato leaves has no effect on the size of the meal taken when the beetles first encounter the leaves, even though beetles of this same strain do not eat tomato[28]. In a second example, in 6-h choice tests with larvae of the armyworm, *Pseudaletia unipuncta,* there was no evidence that nicotine had any deterrent effects, but on first encounter with nicotine-treated leaf material rejection was much more frequent than in controls[52]. It is apparent that in the field the relationship of the insects to plants containing these chemicals might not be what we would expect from the results of long term laboratory studies.

Direct observations are needed for a proper understanding of the behavior of the insects as it affects ecology, and in the past few years two areas of general interest have emerged as a result of such behavioral studies. These are: first, the importance of leaf-surface chemicals and, second, the role of learning in host-plant selection by phytophagous insects. These two topics will be briefly reviewed.

The effects of leaf-surface chemicals on behavior

The role of chemicals on the leaf surface in host-plant selection has previously been reviewed by Chapman[18]

and Woodhead and Chapman[59], but sufficient new information has accumulated since the last of these to warrant a further reappraisal. Our review does not include olfaction or chemicals produced by glandular trichomes (see papers in Juniper and Southwood[34]), and we restrict our comments to behavior associated with feeding.

Behavior at the leaf surface
Exploratory behavior. Critical observations of a number of different insect species show that before feeding on a plant they explore its surface. Several grasshoppers[3, 9, 19] and the beetle, *L. decemlineata*[27], explore the surface with the palps, bringing the contact chemoreceptors at their tips into a succession of brief contacts. Following palpation, grasshoppers sometimes make a test bite, apparently without removing tissue, and the beetle compresses the leaf with its mandibles, sometimes expressing leaf sap. Blaney and Simmonds[12] describe the behavioral sequence involved in plant acceptance by larvae of the African armyworm, *Spodoptera exempta*. The caterpillar first palpates the leaf surface with the maxillary palps and styloconic sensilla, then bites, nibbles and samples before finally going on to feed. In Homoptera, which lack palps, the tip of the proboscis may be used instead. The whitefly, *Parabemisia myricae,* rubs the apex of the rostrum over the surface of a leaf, often just before probing[55], and the brown planthopper, *Nilaparvata lugens,* also explores the leaf surface before probing, tapping with the tip of the labium[20] which bears a number of chemoreceptors. Aphids also tap the surface with the tip of the labium, but in this case no chemoreceptors are present. However, it has been noted that they eject and withdraw small quantities of watery saliva which may enable them to detect chemicals on the leaf surface with the receptors in the cibarial cavity[42].
In addition, insects have contact chemoreceptors on their tarsi so that the mere fact of standing or walking on a leaf will provide them with information concerning the chemical nature of its surface. In grasshoppers there is evidence that information perceived by the tarsi can have an effect on host-plant selection[19, 50] (and unpublished observations). The antennae may also be important and will certainly perceive odors in the layer of air above the leaf surface.
This exploratory behavior is difficult to see, largely because of the very small size of most insects, but wherever it has been looked for it has been seen. The probability is that all phytophagous insects make some sensory exploration of the leaf surface as a prelude to biting.

Exploratory behavior and food selection. Acrididae. Blaney and Chapman[9] first drew attention to the fact that acridids sometimes rejected food without biting it. They showed that if nymphs of the migratory locust, *Locusta migratoria,* were offered a leaf of the non-host, *Bellis perennis* (Asteraceae), after an average interfeed period following a meal on wheat, more than 60% of the

insects rejected the leaf following palpation, i.e. without biting. Similar results were subsequently obtained with a number of unacceptable plants and with a number of different acridid species[2, 3, 18] (and unpublished observations). In some cases partial removal of the epicuticular wax reduced the occurrence of rejection at palpation and insects tended to go on to bite the leaves before rejecting them. The response to some other plant species, however, was unaffected by the treatment[28]. Removal of the surface resin of creosote bush, *Larrea tridentata* (Zygophyllaceae), with an ether wash increased the palatability of the leaves to *Cibolacris parviceps*[48]. The extraction times in these experiments were very short, but nevertheless these results must be treated with caution, since the solvents may have affected the leaves in ways other than the removal of wax. *Bootettix argentatus* rejected all non-hosts (11 species tested) without biting. In this case tarsal contact, or olfaction, even without palpation was often sufficient to cause the insects to leave the plants. This was still true after 20 h without food[19].
Host plant species are also sometimes rejected at palpation. This has been demonstrated in several of the papers cited above, by Blaney et al.[14] in both *L. migratoria* and *Schistocerca gregaria,* the desert locust, and by Woodhead[57] in *L. migratoria.* Immature sorghum is not palatable to this species and was commonly rejected following palpation, but after removal of the suface wax most insects went on to bite. Mature sorghum was almost invariably eaten. Fourth instar nymphs of *Schistocerca gregaria* usually rejected turgid leaves of *Taraxacum officinale* (Asteraceae) at palpation, but if the leaves became flaccid they were readily accepted[5].
In an important study, Blaney and Simmonds[10] showed that the response of *L. migratoria* to at least some non-host plants depended on experience. When the insects first encountered nagweed, *Senecio,* or cabbage, *Brassica,* they usually bit them before rejecting them, but after three of four encounters the plants were rejected at palpation. Switching from one species of *Senecio* to another resulted in a partial return to biting before rejecting, but a switch from *Senecio* to *Brassica* led to an almost complete return to biting behavior.
Homoptera. Klingauf and co-workers[35, 36] demonstrated that the settling behavior of the aphids, *Acyrthosiphon pisum* and *Rhopalosiphum insertum,* was affected by the quality of the plant surface; on leaves of non-hosts the duration of wandering was longer than on those of a host plant. Walker[54] showed that whiteflies, *Parabemisia myricae,* distinguish between young and old leaves of lemon before probing. On young leaves 90% of contacts led directly to probing, whereas on old leaves only 30% did so. On isolated cuticles this distinction did not occur. However, the duration of probes into sachets containing sucrose was strongly influenced by such cuticles; much longer probes were made into sachets covered with the cuticle of young leaves than into sachets with cuticle from old leaves. The latter also reduced probing time relative

to a parafilm sachet[55]. The exploratory behavior of the planthopper, *Nilaparvata lugens,* on a susceptible rice variety is usually followed by probing, but on two resistant varieties the insects commonly did not probe, but lifted the labium away from the plant surface before going on to a further period of exploration[20].

Lepidoptera. When larvae of *Spodoptera exempta* were initially presented with cotton or cabbage, both non-hosts, rejection occurred most often after nibbling or sampling[11], but after several successive presentations some larvae rejected at palpation.

Coleoptera. Harrison[27] showed that adult *Leptinotarsa decemlineata,* on first encountering a leaf, walked over the surface examining it with the maxillary palps. This phase of behavior lasted longer on leaves that were subsequently rejected or eaten in smaller quantities than on the principal host plant and it was at this stage that discrimination between hosts of different acceptabilities was made. Subsequently the beetle macerated the leaf with its mandibles, expressing a drop of plant sap from an acceptable plant, but apparently not doing so on plants that were not eaten. However, on potato leaves that had been infused with a deterrent non-host alkaloid, atropine, the duration of the maceration period was extended and beetles often rejected the leaf[28]. The positive information provided by chemicals on the surface of acceptable plants thus seems to be confirmed by tasting the internal fluids before feeding begins. This behavior was exhibited by beetles that, as adults, had no previous experience of the plants and which, as larvae, had experienced only the normal host plant for that insect race.

Adult pea weevils, *Sitona lineatus,* were affected by alkane fractions of extracts of host and non-host plants. The number of feeding probes was higher on paper treated with the host extract in a choice test[37].

Conclusions. These examples offer ample evidence that phytophagous insects do react to features of the surface environment of leaves, but they give no indication of whether the response is mediated by the physical properties of the leaf surface, by odors just above the leaf surface, or by chemicals on the surface.

Rejection of plants by grasshoppers before biting is commonly observed only if they are not deprived of food. With periods of deprivation in excess of the normal interfeed periods an increasing proportion of the insects will bite a non-host before rejecting it[3,9]. Consequently the importance of palpation is overlooked in many studies where grasshoppers are deprived of food for many hours before an experiment to ensure that they will eat. This may also apply to other insects, but no data are available. Doubt is sometimes expressed about the ability of insects to perceive substances on the leaf surface. Many of these substances are non-polar and the leaf surface is usually dry. How can these chemicals move from the leaf surface to the acceptor sites on the dendrite membrane? Is it possible that during exploratory activities the sensilla penetrate the leaf surface and are, in fact, stimulated by

the fluid contents of the leaf? This seems highly unlikely given the delicate structure of most contact chemoreceptors, and those on the palps and pulvillar pads of Acrididae are set in flexible membranes. These arrangements seem to be an adaptation to avoid excessively strong contact with the surface. Two investigations have specifically examined the question of penetration. Blaney and Chapman[9], in a scanning electron microscope study, failed to find any evidence of damage to a leaf surface after palpation, and Traynier and Hines[51], using a chemical method, found no evidence of damage to the surface of *Brassica* following drumming by female cabbage butterflies, *Pieris rapae,* before oviposition. Thus there is good reason to believe that insects do have the capacity to perceive non-polar substances on the dry leaf surface.

Evidence for responses to leaf-surface chemicals

Evidence that insects respond to chemicals on the leaf surface is obtained from experiments using surface extracts of leaves or pure chemicals that are known to occur on leaf surfaces. Two types of assay have been used in these experiments: a true behavioral assay in which the behavior of the insect has been observed, and assays that measure the effects of surface chemicals on amounts eaten over a period. The latter type of assay may demonstrate that a substance is perceived by an insect and does affect feeding; it can only be inferred that it has similar effects when it is on the leaf surface.

Behavioral assays. Behavioral assays with *L. migratoria* showed that non-polar extracts of the leaf-surface of *Poa annua,* a highly acceptable host plant, increased the amount of biting on a strip of filter paper compared with solvent-treated controls[6] but a surface extract of *Bellis* did not reduce the amount of biting even though the intact leaf was frequently rejected at palpation[9]. A chloroform extract of the leaf-surface resin of *Larrea,* the host plant of *Bootettix argentatus,* stimulated the insects to palpate and bite on filter paper (control insects did not usually palpate) and also made them bite and nibble when applied to the leaves of a non-host that was normally completely rejected[19].

Amongst aphids, the wax of *Vicia faba,* a host plant, stimulated movement of the pea aphid, *Acyrthosiphon pisum,* from the upper to the lower surface of a leaf, but the wax of *Brassica,* a non-host, had no effect. Further testing of fractions of the extract of the host wax showed that the activity was present in the alkane fraction. Dominant alkanes were $C_{27} - C_{33}$; in *Brassica* the dominant alkane was C_{29}. It is also reported that fatty acids usually had a deterrent effect[37]. Short-chain $(C_8 - C_{13})$ monocarboxylic acids inhibited settling by the green peach aphid, *Myzus persicae*[26,45], while longer chain lengths and dicarboxylic acids encouraged it[26]. There is, as yet, no evidence that these chemicals have these effects on leaf surfaces although they are often present.

Woodhead and Padgham[60] showed that the application of epicuticular wax from a rice variety that was resistant

to *Nilaparvata lugens* to the surface of a susceptible rice variety increased the level of activity of the insects to that normally associated with resistant plants. Fractionation of the wax revealed that the activity was in fractions containing hydrocarbons and carbonyl compounds. The resistant variety has a higher proportion of short- to long-chain compounds. The increase in activity associated with the wax was often associated with a move away from the leaf sheath, where feeding normally occurs, out on to the leaf blades.

Long-term assays. Long-term feeding assays of surface extracts and chemicals also indicate the occurrence of both phagostimulatory and deterrent effects. Surface extracts of 11 non-hosts tested with *Chorthippus parallelus*[3] and 11 non-hosts with *Bootettix argentatus*[19], were deterrent when tested on artificial substrates in all but three instances. On the other hand, chloroform surface extracts of normally acceptable plants had variable effects. Of extracts of six grasses tested against *C. parallelus*, only one was phagostimulatory and two were deterrent. The chloroform extract of *Larrea* surface resin was phagostimulatory for *B. argentatus* but a methanol extract was deterrent for this species and also for the grasshoppers, *Ligurotettix coquilletti* and *Cibolacris parviceps*, both of which will feed on the plant. Nordihydroguaiaretic acid (NDGA), which is a major component of the resin of *Larrea*, is phagostimulatory for *B. argentatus* over a wide range of concentrations, but deterrent to the other two species at naturally occurring concentrations[19].

The effects of variations in wax components of different varieties of sorghum on the feeding response of *L. migratoria* has been investigated, notably by Woodhead[56,57]. Surface extracts containing the non-polar compounds of two varieties were deterrent when made from plants up to 14 days old, but extracts from older plants had no effect[1]. Deterrence was produced by fractions containing the n-alkanes, esters and *p*-hydroxybenzaldehyde[56]. Amongst the alkanes only the C_{19}, C_{21} and C_{23} compounds had a significant effect, while others across the range $C_{18} - C_{28}$ had none[57].

Klingauf[35] incorporated an aqueous surface extract of apple leaves into parafilm sachets and determined that *R. insertum* probed for longer than in controls with water alone. This extract contained the phenolic glycoside, phlorizin, which itself increased the duration of probing by *R. insertum* and *Aphis pomi*, but had the opposite effect on *A. pisum* for which apple is a non-host. On the other hand, *A. pisum* probed for longer into sachets containing n-alkanes from the surface of *Vicia* and also in response to the C_{32} alkane; C_{23} and C_{28} alkanes were ineffective[36]. Jordens-Rottger[33] has shown that amino acids present in very low concentrations on the leaf surfaces of both host and non-host plants stimulated feeding by *Aphis fabae*.

Mori[43] examined the effects of whole leaf extracts of mulberry on the selection of diets by newly hatched larvae of the silkworm, *Bombyx mori*. A fraction containing

the major alcohols from the surface waxes was the most active and in further experiments a strong preference for, and phagostimulatory effect by, the C_{26} and C_{28} alcohols was demonstrated. A marginal effect was produced by the C_{30} alcohol, but the $C_{20,22}$ and C_{24} alcohols had no positive effect.

In long-term assays with spruce budworm, *Choristoneura fumiferana*, it was shown that extracts of the epicuticular wax of both white spure and balsam fir, two normal hosts, stimulated feeding[40]. This study also showed that the degree of stimulation by the wax varied from tree to tree within a species and also that the extracts made at different times from a single tree varied in their effects. The differences between individual white spruce trees could be attributed to a single fraction containing diterpene acids, dehydroabietic acid, diols and a fatty acid and alcohol. Three fractions were implicated in the difference in response to two individual balsam fir trees.

Unidentified non-polar compounds from the leaf surfaces of a number of host-plant species have been shown to stimulate feeding by the tobacco hornworm, *Manduca sexta*, in long-term tests while extracts from a non-host, *Canna*, had no effect[16]. Removal of the wax decreased the acceptability of host plants, and increased acceptability of *Canna*. These results demonstrate the presence of phagostimulatory compounds in the wax of the host plants and suggest a deterrent in the wax of *Canna*. However, no deterrent effect was produced by the surface extract of this plant.

Amongst the Coleoptera, McKibben et al.[41] have shown that a number of esters from the surface wax of cotton buds and anthers are phagostimulants for adult boll weevils, *Anthonomus grandis*. The two most important are a geranylgeraniol with a C_{22} acid moiety and a phytol with a C_{12} acid moiety.

From these data it is clear that chemicals from the leaf surface commonly contribute to the acceptability of a plant to phytophagous insects. Effects have been found in every case where a specific investigation of the leaf surface has been carried out and we believe that it may be a general phenomenon. Much of the information is, however, not associated with observations on the behavior of the insects on plants and without this no firm conclusions can be drawn about the importance of these chemicals in host-plant selection.

Occurrence in the field

Because most of the behavioral observations have been made in the laboratory, the ecological relevance of this behavior can only be inferred. Do the same things happen in the field?

In the laboratory, behavioral responses to surface features are rarely seen unless the insect is undisturbed and has not been deprived of food for a long period beforehand. In the field there are times when unsuitable environmental conditions, especially temperature, may result in extended periods without food (see, for example,

Chapman[17]) and under these circumstances the subsequent selection of food may be less particular and surface features may not be involved. When environmental conditions are suitable, however, it is likely that the insects feed in regular patterns and do use surface features in identifying food plants.

The only example of rejection of a potential food-plant in the field is the observation by Bernays et al.[7] that nymphs of *L. migratoria* rejected non-hosts without biting them. In addition several studies (e.g. Woodhead and Padgham[60]) report that rejection of a leaf after surface contact was followed by movements that might be expected to take the insect away from the plant in the field.

Surface chemistry and host-plant selection

Some studies have demonstrated that plant secondary compounds occurring on the leaf surface may act as phagostimulants for adapted species or as deterrents for those less well adapted. This is the case, for example, with NDGA from the surface of *Larrea*[19] and phlorizin on the surface of apple leaves[35]. In most cases, however, the active fractions or compounds have proved to be non-polar and the chemicals involved are of widespread occurrence. They may have phagostimulatory or deterrent effects. How can such chemicals contribute to host-plant selection?

Amongst oligophagous species intensive studies have sometimes failed to reveal specific phagostimulants that wholly account for the host plant range of the insect. This is true, for example, of *L. migratoria, Bombyx mori, Manduca exta, Leptinotarsa decemlineata* and *Anthonomus grandis,* despite the fact that for all expect *L. migratoria* the host plants are characterized by specific secondary compounds. In all these insects a positive role for the surface wax has been established, but where the compounds have been identified they are not restricted to the host taxa. How, then, is specificity achieved in these insects?

There is ample evidence that most, probably all, phytophagous insects are inhibited from feeding by secondary chemicals in non-host plants. The effect of this is to restrict the insect to plants that lack feeding deterrents. Feeding will be initiated by commonly occurring phagostimulants, including those on the plant surface, and will continue provided these are not associated with deterrents. No specific phagostimulant is required. That this is so is demonstrated by the development of artificial diets without any plant-specific compounds for a number of these insects.

We do not suggest that other factors are unimportant in host plant recognition. Olfaction is sometimes obviously important in attraction, but so far there is little evidence of a role in the final stages of recognition (but see, for example, de Boer and Hanson[15]); in holometabolous insects the host-selection behavior of the parent insects may have a major role. Nor do we suggest that oligophagous insects never make use of phagostimulants

that are characteristic of the host; *Pieris* is an obvious example of one that does[39]. But we do believe that there is good evidence that host-plant recognition by some oligophagous insects does not depend on the presence of secondary chemicals characteristic of the host taxa, and in these cases chemicals on the leaf surface are important phagostimulants.

The role of experience

The work of Blaney and Simmonds on a locust[10] and a caterpillar[11] shows that experience can be an important factor in the response of insects to plant surface chemicals. The question arises: is this always true?

It is certainly true that in many experiments where insects have been shown to respond to surface chemicals they had not previously experienced the plants with which they were tested. For example, the acridids tested on a non-host, *Bellis,* by Blaney and Chapman[9] had not previously experienced it; the *L. decemlineata* observed by Harrison[27] had no previous experience of any plants as adults. Rejection in these cases seems to indicate innate responses, but it is possible even here that experience is important. Although the insects had not experienced these particular foods they cannot be regarded as naive because they may have developed a 'memory template' of the taste of food on which they had previously fed (e.g., *L. decemlineata* as larvae), and novel foods may be judged by their match to this template. We do not know if this occurs, but the possibility cannot be ignored. Firm evidence against a learning process can only be obtained from a study of newly hatched, completely naive insects. The only relevant experiments on food selection using newly hatched insects with no previous experience of food are those of Mori[43]. These demonstrate unequivocally that larval *B. mori* exhibit an innate positive response to plant surface alcohols. Although they are not directly concerned with feeding, the observations and experiments of Bernays et al.[8] and Woodhead[58] on the climbing behavior of newly hatched larvae of the stemborer, *Chilo partellus,* also show that they have an innate positive response to components of the surface wax of the host. Thus there is some evidence of innate effects of surface chemicals that have positive effects on behavior. There is as yet no direct evidence of an innate *deterrent* response to commonly occurring surface chemicals. This is important to establish since it is with respect to deterrence that the observations of Blaney and Simmonds[10,11] are directly relevant. Intuitively, it seems more logical that deterrent responses that involve common surface chemicals should be learned since otherwise there is the risk, as Blaney et al.[14] suggest, that palatable plants would be avoided.

Learning in host-plant selection

The second major aspect of food selection behavior to which behavioral studies have contributed is the role of

experience. In reviewing this topic Jermy[30] discusses three separate phenomena: induction of preference, habituation, and food aversion learning. Although induction of feeding preference has been recognized for some time and is known to occur in over 30 species belonging to all the major taxa of phytophagous insects except Orthoptera[30], there has been little behavioral work on this topic. Here we will discuss habituation and associative learning, including aversion learning, since it is in these areas that the study of behavior has made its greatest impact. Although much of the recent work has focussed on oviposition[44] new studies on the impact of learning on food selection warrant a brief review.

Habituation
Habituation is the waning of a response as a result of repeated presentation of a stimulus. The first clear demonstration of this in relation to the feeding behavior of insects is probably by Gill[25] who showed that *Schistocerca gregaria* ate progressively more of a diet containing low levels of azadirachtin, a deterrent for this species, the longer they were exposed to it. This was probably not simply a matter of increasing starvation because the insects were given some fresh uncontaminated food daily. Subsequently, Jermy et al.[31] carried out experiments in which they specifically investigated the possibility of habituation to feeding deterrents by two acridids and two lepidopterans. These experiments were designed to eliminate any effects of deprivation, which alone will tend to increase the tolerance of deterrents. Quinine and strychnine in a semisynthetic diet were given to the caterpillars, and nicotine hydrogen tartrate (NHT) was applied to the surface of sorghum leaves for the acridids. All the insects exhibited some degree of habituation; those with experience of the deterrent ate more contaminated food than control insects with no previous experience.

In detailed behavioral observations on *S. gregaria*[50] the insects at first rejected a leaf coated with NHT on tarsal contact alone. After about 4 h they started to bite before rejecting and then, after 7 or 8 h, they took a small meal. In analyzing these first meals we presume that the sensory input signalling rejection is offset by the internal signals arising from the state of deprivation. On subsequent days, however, the first meal was taken progressively earlier in the day, until by day 4 it occurred in the first hour of the test. Between test periods the insects had access to an ample supply of good food, so the change from day to day was not a consequence of increasing deprivation even though the initial changes in the first day can be attributed to this.

A similar picture emerges in the response of *Spodoptera frugiperda* to leaf discs treated with aristolochic acid[47] although the authors call the process 'desensitization'. When the insects were effectively forced to feed on the test compound through a shortage of untreated food they subsequently began to feed on treated discs earlier than a control group that had previously eaten hardly any of the test compound. Habituation to NHT by *Pseudaletia unipuncta* over a 6-h period has also been demonstrated[52]. When first presented with treated leaf discs larvae often rejected them, but after 6 h there was no reduction in feeding compared with controls.

The question as to whether an insect can habituate to a complex of stimuli including a mixture of deterrents such as might occur in non-host plants, was tested by Jermy et al.[32] using larvae of a polyphagous strain of *Mamestra brassicae*. No habituation occurred on the majority of non-host plants, but on potato, on which there was a great deal of individual variation, about 10% of the insects did habituate. This was evident even after a single exposure of 17 h.

Is habituation likely to occur in the field? Presumably in many cases the initial rejection response, as observed in the experiments of Szentesi and Bernays[50] and Usher et al.[52], would result in the insect moving to another plant. It is conceivable, however, that a small, relatively immobile insect, or one in a large patch of a relatively unpalatable plant, could remain in contact with a particular plant species for long enough for habituation to occur. There is currently no evidence of this occurring, but it is possible that it is a regular phenomenon in newly hatched larvae from eggs laid on marginally palatable hosts.

Associative learning. Blaney an his co-workers have provided evidence that both acridids and lepidopterans are able to associate the taste of leaf surface chemicals with internal constituents of the leaves. The insects under study were shown to palpate the surfaces of non-hosts and commonly bite into them on first encounter before rejection, while on subsequent encounters there was a significant increasing tendency to reject them on palpation alone[10 – 13]. The possibility that this resulted from an overall depression of feeding level was discounted on the grounds that a second unrelated non-host, presented after biting stopped on the first non-host, once again elicited the initial biting activity, followed by the decline. There is a presumed association between sensations at the leaf surface and sensations after biting. It remains a possibility that the mechanism is one of sensitization rather than associative learning, but the change is in any case interesting in view of the possible increased speed with which whole plants may be tested and rejected.

Food aversion learning, in which the organism associates a particular taste with a noxious physiological effect, has been clearly demonstrated experimentally in the grasshopper, *Schistocerca americana*[4]. The association was produced by injecting a toxic compound into the hemocoel immediately after a meal so that the subsequent toxemia was associated with the food just eaten. Subsequently this food was only eaten in very small amounts or was completely rejected; other food plants remained acceptable. The compounds tested were nicotine hydrogen tartrate, quinine chloride, coumarin, ouabain and lithium chloride. Aversive behavior was induced by each of the chemicals but the association was only clear when

the food was not maximally acceptable in the first instance as was found to be the case with spinach.

Possible food aversion learning to gossypol and chrysanthemum monocarboxylic acid ethylester was also demonstrated in *Spodoptera frugiperda* by Raffa[46]. He concludes, on the basis of tests with 15 chemicals, that innate feeding preferences generally override the effects of experience, but his experiments are not a valid test of aversion learning because in the final test untreated corn was used; because of the design of the experiments, the taste of untreated corn had never been associated with any toxemia.

In the experiments of Bernays and Lee the injection of toxin was designed to simulate the effects of absorption from the gut after eating food containing a toxin. In experiments with two polyphagous caterpillars Dethier[22] demonstrated aversion learning following ingestion of a toxic plant. *Petunia* is a preferred food plant of the larvae of *Diacrisia virginica* and *Estigmene congrua*, but its ingestion is followed by observable 'illness'. After recovery, the larvae eat less *Petunia* and more of an alternative food plant that was previously less preferred. Although these experiments lack the critical observations that the insects do really reject *Petunia* they do appear to show that food aversion learning of a plant within the host range of the insect can occur. No evidence of food aversion learning was obtained with the oligophagous larvae of *Manduca sexta*[24].

Another example of what is believed to be aversion learning of a plant has been found with *Schistocerca americana*. Nymphs of this species were invariably found to eat less spinach in successive meals and finally to reject it. Although no symptoms of toxicity were observed, this food did not support development, and the change is interpreted as aversion learning. Direct feedbacks were discounted: spinach placed in the gut without its being tasted did not cause the change in behavior seen after actual feeding[38].

There is an indication that caterpillars of the arctiid *Diacrisia virginica* learned to reject *Petunia* in a field setting, after they were observed to regurgitate and show other symptoms of toxicity following confinement on this plant[23]. The data are based on the distributions of insects after set intervals rather than on behavioral observations but do suggest aversion learning.

Declines in palatability have been observed with several foods that are only moderately acceptable to *S. americana* (Lee, unpublished observations), and it is conceivable that food aversion learning is an important feature of polyphagy at least in acridids. Work on slugs[21] shows that the malaise involved in food aversion learning need not be due to toxic compounds, but may be induced by an imbalance of nutrients. There is now increasing evidence, principally with artificial diets[49, 53], that insects can adjust their food intake to improve nutrient balance. It is possible that this is dependant on sequential aversion learning ensuring dietary switching.

Future work

We believe that the aspects of behavior discussed in this review may be of considerable importance in host-plant selection by phytophagous insects, but this will be established with certainty only through the critical observation of behavior. Two aspects, in particular, need attention: 1. the study of the behavior of newly hatched insects when they first encounter a plant, and the effects of this first encounter on subsequent behavior; and 2. observations of behavior in the field. Both are difficult because of the small size of the insects and the ease with which many species are disturbed, but they are essential to gain a good understanding of the basis of chemical ecology.

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