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Cover drawing: Feeding patterns of Colorado beetle larvae on potato leaves (see J. de Wilde, 1958. Host plant selection in the Colorado beetle larva.
Entomologia experimentalis et applicata 1:14-22).
Drawn by W.C.T. Middelplaats, Agricultural University, Wageningen.

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Introduction

Only fairly recently have entomologists begun to realize that insects possess intimate and subtle relationships with their host plants. R. Painter's book 'Insect resistance in crop plants', published in 1951, reflects the beginning of interest in insect-plant interactions. In the same year, Professor de Wilde, at the 9th International Congress of Entomology at Amsterdam, organized a symposium on relations between insects and host plants and invited home a few of the early specialists in this field, including V.G. Dethier, G.S. Fraenkel, J.S. Kennedy, A.J. Thornsteinson and P. Grison. Close friendships and scientific collaboration were established for years to come.

In 1958, de Wilde organized a symposium on this subject at Wageningen, attended by about 30 scientists active in the field. The proceedings formed the first issue of Entomologia experimentalis et applicata. The 2nd symposium took place in 1969, maintaining the regular contacts for workers in this field: the 3rd at Tihany in Hungary was organized by T. Jermy and the 4th at Slough in 1978 by R.F. Chapman and E.A. Bernays. There it was decided that it would be appropriate to hold the 5th symposium at Wageningen, to coincide with the retirement of Professor de Wilde.

The participants of the 5th meeting thought it appropriate to dedicate these proceedings to the founder of this series: Jan de Wilde.

L.M. Schoonhoven

Physiology

The insect on the plant — a closer look

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Abstract

The characteristics of insects which may affect their interactions with plants are considered, with a view to developing new ideas, and identifying particular areas of work which need further study. Size, and some of its important repercussions is discussed, including the problem of orientation to odour sources, the significance of the surface, and the ability to detoxify allelochemics. Poikilothermy and its correlates in feeding requirements is briefly discussed. Finally the presence of a sclerotized exoskeleton is discussed with particular relevance to the need for aromatic compounds in the diet for cross-linking purposes in the cuticle. It is demonstrated that in plant foods, protein and hence aromatic amino acids are often in short supply and that the shortage of protein exerts its most profound influence through the need for phenylalanine. A large protein intake is required to preferentially extract aromatic elements, and some insects make direct use of plant phenols.

Key-words: nutrition, toxicity, alkaloids, cuticle, proteins, amino acids, phenylalanine, phenols, *Schistocerca gregaria, Anacridium melanorhodon*

The last decade has seen a snowballing of interest and work on insect-plant interactions, and even in the past two years there have been well over 2 000 publications on the topic. Behavioural and sensory aspects have been well covered, from both causal and functional points of view, so that this 'closer look' is an attempt to stand back from all the theories and the accumulation of detailed information, and to look again at the nature of insects. Perhaps the examination of some peculiarities of insects can give us new leads in the understanding of insect-plant relations, and suggest fruitful areas for further work.

Size

Among the major herbivore groups, the insects are most obviously different in terms of size. This has several important repercussions. Firstly, the discontinuity of natural odours, together with the variations in air turbulence has much greater significance for small organisms than for large ones. Stable odour gradients do not exist in the natural environment. This has recently been well demonstrated from a

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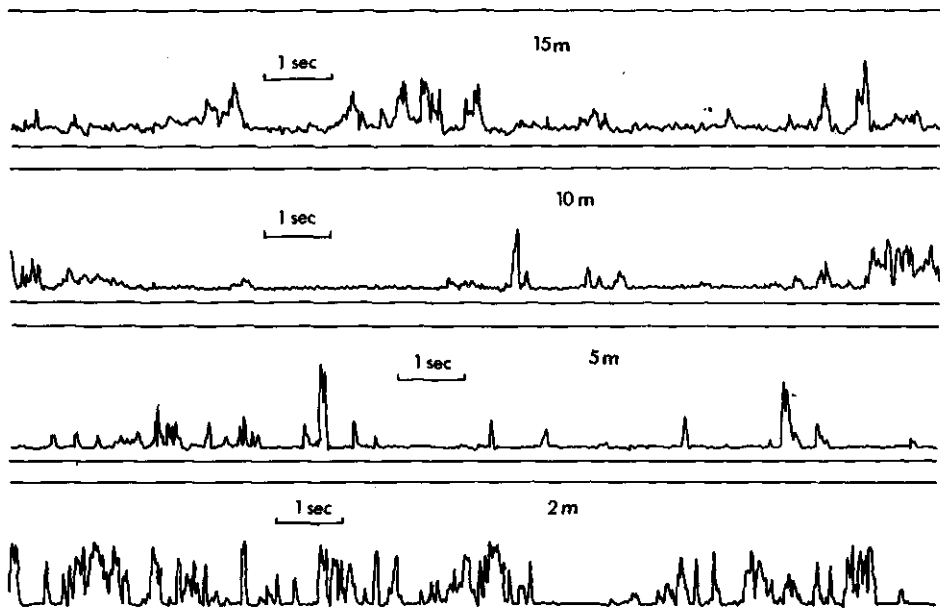
Fig. 1. Discontinuity of ion distribution at different distances downwind from an ioniser which emits continuously (after Murliss & Jones, 1981).

point source by Murliss & Jones (1981) (Figure 1). In order to orient the insect must either depend on a complex temporal analysis of the signals reaching it, or, as is probably usual, depend on anemotaxis, the volley of signals at different times serving merely to maintain its response (Kennedy, 1977). Larger animals, sampling larger volumes of air have at least the opportunity of perceiving odour gradients.

Having arrived at the plant surface, the size of the insect becomes even more significant. It inevitably means that the leaf surface dominates the environment of an insect on it, and the nature of this surface is consequently of primary importance in its behaviour. Many chemoreceptors, if not the whole insect, will frequently be within the boundary layer, where the range of detectable air-borne plant chemicals is maximal and the concentrations of individual chemicals is several orders of magnitude higher than at a distance of even 1 cm. Thus it is likely that contact chemoreception and close-range olfaction within the leaf boundary layer is especially significant in practice, for insects' food selection. This would also be suggested by relative number and range of sensitivities of contact chemoreceptors in any phytophagous insect (Chapman, 1982). The general importance of the surface topography, and the chemistry on and immediately above the leaf surface, in the behaviour of phytophagous insects was reviewed by Chapman in 1977, and little further work has been done in this difficult field, although there are a few recent studies (e.g. Hamilton et al., 1979; Städler, 1977,1978; Williams et al., 1980). Three excellent studies in this volume serve to demonstrate the complex and important role of the leaf surface (Städler & Buser, p.403; Woodhead, p.375; Blaney & Winstanley,

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p.365). These more careful studies combining chemistry with observations on insects which have not been deprived in an unnatural manner, certainly reinforce the belief that, in spite of the difficulties, the role of plant surfaces demands a major effort in the future.

One of the risks of being a small organism on a plant is that of being eaten by a vertebrate herbivore and it would be adaptively advantageous if there were selection of plants or plant parts avoided by vertebrates. We know that many allelochemics affect both insects and vertebrates, but certainly there are many examples of plants avoided by vertebrates but eaten by insects. We also know that few plant species have notably low incidence of insect herbivores, and species diversity is not significantly influenced by plant chemistry per se (Lawton, 1978; Strong, 1979). Also, many insect herbivores sequester secondary plant metabolites for their own protection. This argues for a remarkable class plasticity and adaptability within the Insecta, and perhaps a greater sensitivity to potential toxins, of the vertebrates, which may be unwitting predators. Are insects particularly insensitive to allelochemics?

Detoxification capacity

Insects which have been tested behaviourally with a wide range of plant extracts are usually inhibited from feeding on extracts of non-hosts (e.g. Bernays & Chapman, 1977; Jermy, 1966). Such effects may indicate toxicity but may simply signal plants to which the insect is less suited for other reasons. Misunderstandings abound in this field of work. For example, toxicity is often quoted as a reason for an effect on a herbivore in bioassays, when deterrence has not been separately measured, and the so-called toxicity is sometimes simply a reduced food intake. Again, reduced food intake may itself lead to reduced utilisation indices, which certainly occurs in caterpillars (Schroeder, 1976). This may in turn lead to a false measure of potential post-ingestional toxicity. Finally, total deterrence may prevent the measure of oral toxicity by feeding experiments. This is common with acridids.

Under starvation or desiccation pressure, insects may be more readily induced to ingest chemicals which have obvious toxic effects, and although the stressed state of the insect will lower the LD_{50} levels, some measure of relative toxicity may be obtained in this way. Ideally, cannulation of materials into the gut, bypassing the sensory system, should give a more accurate measure of potential oral toxicity (Cottee & Mordue, p.379). In spite of the problems there are a few data for comparative purposes. Alkaloids, for example, are a diverse group of allelochemics but if the toxicity to mammals generally and to unspecialised polyphagous insects is compared, there is a remarkable difference (Figure 2), the insects being much less sensitive. Besides this, specialised insects, able to cope with very high concentrations of particular allelochemics are relatively common, while among vertebrates, such specialism is uncommon.

Again, hydrogen cyanide (HCN) is a universal toxin, and is commonly produced by plants, but the sensitivity to HCN released in the gut from the plant glycosides shows that mammals tend to be over one hundred times more sensitive than generalised phytophagous insects (Table 1). If specialised feeders were considered the dif-

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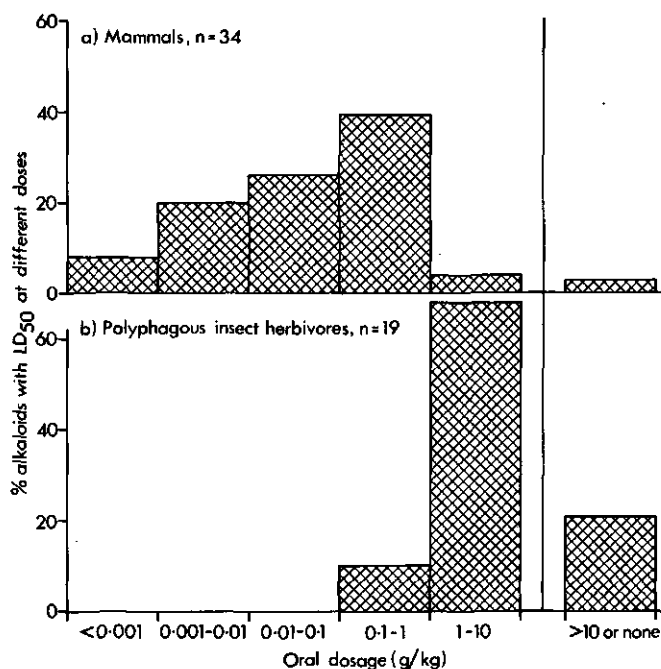
Fig. 2. Alkaloid toxicity levels for unspecialised plant-feeding mammals and unspecialised insects, shown as the percentage of toxic alkaloids having the LD_{50} in the different ranges (mammals compiled from the Merck Index, 1968; phytophagous insects estimated, recalculated or compiled from Bernays, unpubl.; Brattsten, 1977; Cottee, unpubl.; Harley & Thorsteinson, 1967).

ference would be considerably greater. Moreover, although gaseous HCN released in the mouthparts of insects may be deterrent (Woodhead & Bernays, 1978), searches for insect preference of, or selective grazing on cyanogenic strains of species compared with non-cyanogenic strains have only occasionally been successful, and then only where HCN release rates are high and cause behavioural deterrence (reviewed by Bernays, 1982).

Phenols are difficult to compare because many of the excellent studies carried out by Reese & Beck and others, generally involve the use of artificial diets, with the problem of the diets themselves being affected by the phenols, reducing their quality, before ingestion is completed. Many phenolic compounds are very reactive, and the problem is most severe with this chemical class, so interpretation of severe effects on insects is very difficult. Where tannins, for example, have been tested on natural leaf material, the effects on insects have been considerably less marked than where artificial media have been used (Bernays, 1981a).

Why are insects less sensitive than herbivorous vertebrates? At least in some cases special physiological mechanisms endowing the insects with a degree of tolerance to plant secondary chemicals do exist. For example the peritrophic membrane has been shown to selectively adsorb some macromolecules (Bernays, 1981b; Bernays & Chamberlain, 1980), and among acridids, the more polyphagous species have much

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Table 1. Oral toxicity of HCN in some mammals and some poly-phagous insect herbivores.

Animal	Oral LD_{50} (mg/kg)	Reference
Dog	0.8–4.0	Merck, 1968; Conn, 1979
Mouse	3.7	Conn, 1979
Rat	5.0	Conn, 1979
Rabbit	2.0	Merck, 1968
Sheep and cattle	2.0	Conn, 1979
General mammalian	0.5–3.5	Conn, 1979
Locusta migratoria	500	Bernays, unpubl.
Zonocerus variegatus	1000	Bernays, 1982
Spodoptera littoralis	800	Bernays, unpubl.
Spodoptera eridania	1500	Brattsten et al., 1982

more substantial peritrophic membranes (Bernays, unpubl.). Secondly, the midgut epithelium, with its high potential for production of mixed function oxidases and other detoxifying enzymes, perhaps provides a more strategically placed defence than the vertebrate liver (Brattsten, 1977,1979). Thirdly, although insects and vertebrates have filtration excretory systems, the insects have in addition the capacity to actively take up certain toxins (Maddrell, 1981). Fourthly, the insect blood/brain barrier appears to be particularly effective, and may protect the nervous system to an unusual degree (Lane & Skaer, 1980). Fifthly, phytophagous insects are relatively insensitive to cardiac glycosides by known mechanisms (Vaughan & Jungreis, 1977), and in any case with a haemocoel, the heart is a less vital organ. There may also be other mechanisms, and certainly there are exceptions.

It is possible, though the data are still too few, that insects are relatively sensitive to certain chemical classes, such as the terpenoids. At present however the balance of evidence points to a very great tolerance and consequent adaptability of insects in a general way. Finally, returning to size, extremely high levels of potential toxins in certain plants, or plant parts, are readily avoided by being small enough to be selective on the small scale.

Poikilothermy

Insects are in strong contrast with the other major present-day herbivores, the mammals, in being poikilothermic, although this characteristic is of course shared with the reptiles. The energy expended in maintaining body temperature in mammals is high and the daily kJ requirements are orders of magnitude greater per unit body weight than for insects at or near their temperature optima (Figure 3). As a consequence of this, the diet of a mammalian herbivore must yield relatively high levels of energy compared with that of a herbivorous insect. Optimal food requirements of mammals have ratios of protein : energy food (expressed as g protein : g glucose) averaging 1 : 8, while work so far with insects feeding on plants, indicate a need for much higher protein levels in the diet and ratios nearer 1 : 2 in spite of the
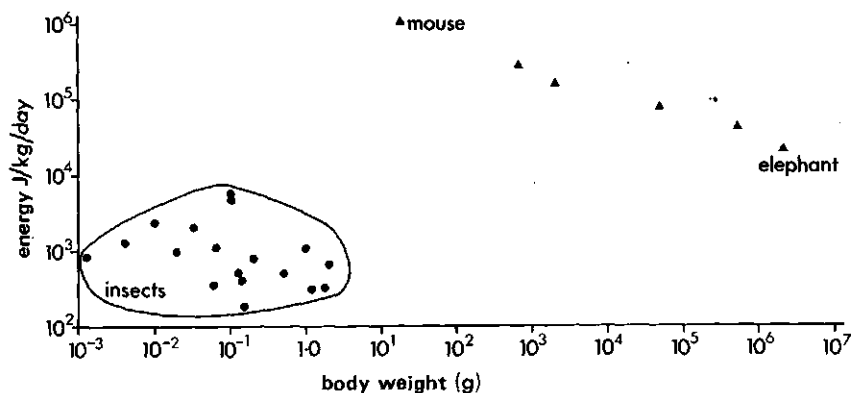
Fig. 3. The daily energy requirements (in joules) for plant-feeding mammals and insects (mammalian values recalculated from Delaney & Happold, 1979; insect values recalculated from values given in Chapman, 1969; Keister & Buck, 1964; Wigglesworth, 1965). Insect values shown at or near optimal temperatures and between 28 and 35 °C.

very high throughput (Table 2). These figures are calculated on the basis of available fat and carbohydrate for energy by the particular animal, and not on conventional bomb calorimetry which includes polymeric materials of very variable availability, together with nitrogenous materials which may be at a premium for other purposes. Cellulose is commonly available to herbivorous mammals but usually unavailable (i.e. indigestible) to insects, and to compare calorific values for the two groups is unrealistic. Indeed selection for means of dealing with the digestion of cellulose in mammals may be seen as a measure of the pressure for high energy levels in this group.

Protein, of course, can be a limiting factor in the growth of any herbivore, but in

Table 2. Approximate optimal ratios of protein: available carbohydrate plus fat (expressed as g glucose) in the diets of some mammals and some insect herbivores.

Animal	g protein : g glucose	Reference
Human child	1:6	Belle et al., 1959
Human female (pregnant)	1:7	
Cattle	1:7	MAFF, 1971
Calves (very young)	1:4	
Heifers	1:7	Williamson & Payne, 1965
Buffalo	1:10	
Goats	1:15	
Silkworm	1:3	Waldbauer, 1968
Silkworm (artificial diet)	2:3	Dadd, 1963
Locust (artificial diet)	1:1	Dadd, 1963
Cabbage butterfly larva	1:1	Slansky, 1974

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mammals, optimal protein levels in the diet tend to be considerably lower than for insects.

Sclerotised exoskeleton

The presence of a light-weight rigid exoskeleton, constructed largely of specialised proteins is one of the basic reasons for the success of insects, and perhaps a consideration of its properties can give us new insights. The rigidity of the cuticle is determined by the number of cross-links between protein molecules, and for this insects invariably employ aromatic compounds which contribute up to 20% of its dry weight (Neville, 1975). A wide range of phenolics has been implicated, but in particular 3,4-dihydroxyphenols, which are often metabolised from tyrosine (see Andersen, 1976; Brunet, 1980).

Does this have any special significance for phytophagous insects? The answer is probably yes but the development of the thesis has not been direct, and began with the finding that tannic acid improved the growth rate of *Anacridium melanorhodon* on a suboptimal diet (Bernays et al., 1980). This was in contrast with the usual idea that tannins are plant defence mechanisms (Bernays, 1981a), but more detailed studies revealed that a number of plant phenols and the aromatic amino acid phenylalanine improved the relative growth rate of the nymphs when added to the diet which was relatively low in both protein and simple phenols (Figure 4). Perhaps this suggests a special requirement for phenols.

Anacridium melanorhodon habitually feeds on acacias and other desert shrubs low in protein, but rich in phenols including gallic acid and various tannins. The fate of
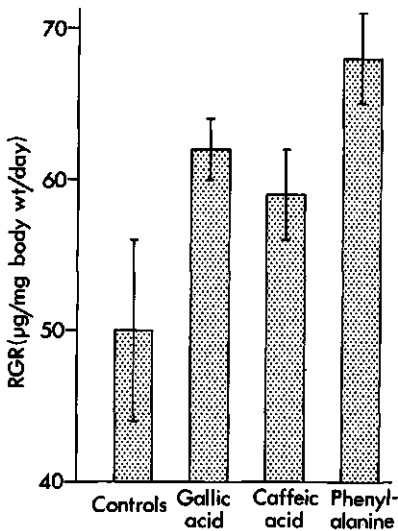
Fig. 4. Relative growth rate of *Anacridium melanorhodon* (sixth instar) on lettuce with different added phenols.
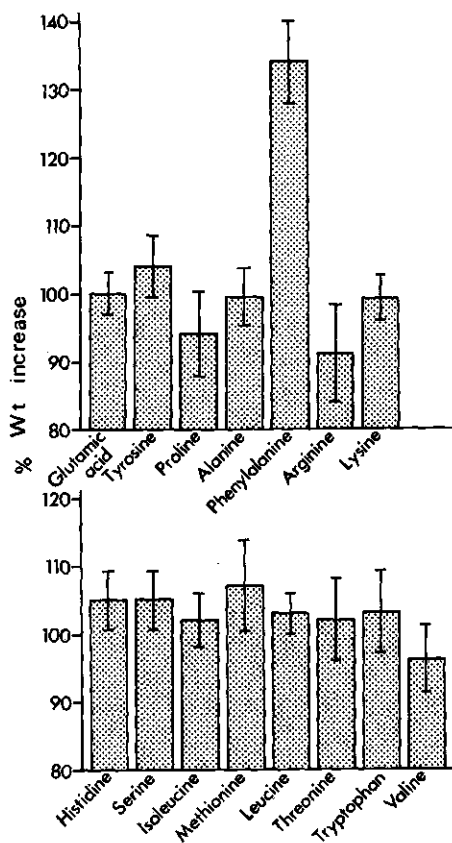
Fig. 5. Percent dry matter increase relative to controls 100, over the fifth instar of *Schistocerca gregaria* having different added amino acids (1 mg/d).

^{14}C-labelled gallic acid ingested by the nymphs was investigated. Most of the phenol taken up from the gut was incorporated into the integument, especially when the insects were near to ecdysis (Bernays & Woodhead, 1982), and the balance of evidence suggested that in this insect, plant phenols, ingested in the normal diet, were used more or less directly in sclerotization of the cuticle, probably replacing or sparing the better-known production of phenols via aromatic amino acids. Was this a general phenomenon or was it restricted to this particular insect? We moved to the study of the polyphagous acridid *Schistocerca gregaria*.

Fifth instar nymphs of *S. gregaria* were fed on a diet of lettuce which was known to be suboptimal, at least in respect of protein levels. Each insect was also provided daily with a disc of glass fibre filter paper containing either 2 mg sucrose or 2 mg sucrose plus 1 mg of an amino acid. Such discs were usually totally ingested, but if not, the quantities of nutrient eaten were calculated. All lettuce intake was measured. There were ten replicates in each case. In no case was consumption of lettuce or length of the instar significantly affected by the treatment, and only phe-

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Table 3. Balance sheet to show ingestion of protein, with and without extra phenylalanine over the fifth instar of *Schistocerca gregaria*, and the apparent value of this amino acid.

	Controls	Test
Number of insects	6	10
Total dry wt of leaf eaten (mean ± S.E. in mg)	1080 ± 45	1076 ± 28
Total mean protein ingested (mg)	325	325
Extra phenylalanine ingested (mg)	0	7.4 ± 0.3
Approx. protein equivalent of 7.4 mg phenylalanine (mg)	–	100
Percent wt increase over instar	82 ± 5	110 ± 5
Protein intake, assuming 7.4 mg phenylalanine ≃ 100 mg protein (mg)	325	425

Cuticle dry wt ≃ 140 mg
Phenolic materials in cuticle: 5% dry wt (Andersen, 1973) ≃ 7 mg

nylalanine significantly increased growth (Figure 5). Clearly there is a special and specific need for phenylalanine over and above any other amino acid, including all the essential amino acids.

It is possible that the specific need for phenylalanine results from the specific need of insects to employ aromatic compounds in the stabilisation of cuticular protein at ecdysis, and the balance sheet for such materials shown in Table 3, emphasises the likelihood of this. Test insects ate a mean of 7.4 mg phenylalanine on top of their lettuce diet, and the resultant dry weight increase over the instar was 34% greater than that of controls. If this quantity of phenylalanine is taken to represent 100 mg of protein, then test insects had the equivalent of 31% extra protein. If phenylalanine is specifically required as shown above, the agreement in these figures is probably not a coincidence. Further, the dry weight of the fully developed fifth instar cuticle is approximately 130 mg, while that of the adult one day after ecdysis is approximately 150 mg, and if Andersen's (1973) value of 5% phenols for the femur is typical of the whole cuticle, then approximately 7 mg phenols is present in both cases. If this is manufactured from phenylalanine, then the value of 7.4 mg phenylalanine ingested by the test insects can be readily understood as a specific need which is directly met, with the saving of up to 100 mg protein from which it must otherwise be extracted. If protein is in excess, the extra phenylalanine would presumably be of little value.

There is considerable additional circumstantial evidence that for phytophagous insects aromatic amino acids are commonly at a premium. Amino nitrogen is notably variable and commonly suboptimal (e.g. McNeill & Southwood, 1978; Mattson, 1980), so that the pressure for aromatic elements is very real. There is every likelihood that:

– Plants can most influence insect growth by having low levels of phenylalanine, or

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that low protein exerts its most profound influence through the need for phenylalanine.

— Some insects make use of plant phenols. Apart from our studies with acridids, there is evidence specially among tree feeding insects where protein is habitually low, that this is so (e.g. Kato, 1978; Hamamura et al., 1966) and even that such compounds have become phagostimulants (e.g. Heron, 1965; Hsiao & Fraenkel, 1968; Meyer & Norris, 1974; see Bernays (1981a) for a discussion of these effects with tannic acid).

Work is required to understand exactly how the phenylalanine is used, and why tyrosine is not used in *Schistocerca gregaria*. The value in growth rate is seen through the first half of the instar (Figure 6) and not just near the time of ecdysis. This may be because at this time there is a major build-up of storage materials and protein utilisation is most efficient (Simpson, 1982). Evidence is accumulating for a dramatic storage of aromatic amino acids at least in some insects during periods of active feeding. In some flies there are storage proteins such as calliphorin, which accumulates in the haemolymph during the last larval instar (Levenbrook & Bauer, 1980). This protein has exceptionally high levels of phenylalanine and tyrosine (Munn et al., 1971). Also *Musca domestica* accumulates phenylalanine in a haemolymph dipeptide during the larval stages (Bodnaryk, 1970,1978), actively taking up phenylalanine in midgut and Malpighian tubule cells. It is released in the cuticle at the time of pupariation (Bodnaryk et al., 1974). Such reservoirs of phe-
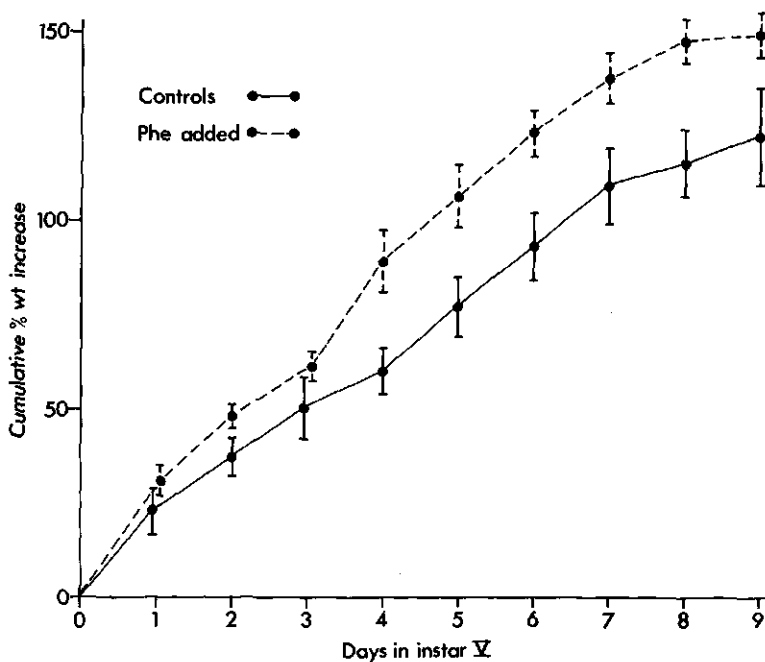
Fig. 6. Cumulative wet weight increase of *Schistocerca gregaria* over the fifth instar, with and without extra 1 mg phenylalanine per day.

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nylalanine and tyrosine have their counterparts in at least some other insects, the quantities being greatest where sclerotization at the following moult is extensive. Thus holometabolous insects have their major requirements and large haemolymph stores in the last larval instar (for pupa plus adult), while insects such as acridids have a considerable need at every moult.

Less work has been done on the protein or peptides stored in phytophagous insects, and whether there is a particularly high level of aromatic amino acids in the proteins which do build up, but there are probably functional analogues of fly calliphorin (e.g. Duhamel & Kunkel, 1978; Gupta, 1977; Kramer et al., 1980; Tojo et al., 1980). Also in caterpillars there are peptides rich in aromatic amino acids which build up especially in the last larval instar and disappear at pupation (Junnikkala, 1976; Sienkiewicz & Piechowska, 1973). Actual amounts of tyrosine or phenylalanine which are accumulated per insect in these studies, and then apparently lost to the cuticle are 5–10 mg.

In summary, phenylalanine is a key nutrient for insects and especially for insects on diet where protein is suboptimal, its importance varying with the phenolic requirement for sclerotization at the subsequent moult. Where plant phenols can replace or spare the aromatic amino acids, they may become phagostimulants.

Conclusions

A consideration of insectan features can give us some new approaches, and certainly points to some major gaps in our knowledge, but the general conclusions to be made from the topics selected here relate to the overriding importance of protein. That it matters has not really been in doubt, but that it matters so particularly for insects compared with other herbivores must influence our thinking on the insect-plant relationship. It should bear considerably on arguments relating to the potential significance of plant nutrient levels, and the possible selection pressures acting upon them. We must also consider more fully the consequences in relation to the behaviour of phytophagous insects.

In the first instance it is vital that insects find high protein sources. Proteins and amino acids however are non-volatile, and the identification of plants which will inevitably be rich in such compounds raises problems. Are there odour signals from protein-rich plant foods? It is possible that among the so-called 'green odours' there may be some correlated with new growth, for example, but we don't know. There may well be visual signals. Similarly, are there signals at the plant surface which are indicative of high protein levels when the insect makes contact? Certainly leaf waxes change with age and quality of the plants and are in a more dynamic state than is usually recognised, but we do not know if such subtleties are interpretable by the insect, or to what extent such changes reflect changes in the protein value of the plant.

It is very common for new plant growth and developing fruits to be relatively rich in protein, while such tissues are also best endowed with toxic secondary metabolites (Bernays, 1982). Such foods are favoured by insects and in particular by specialists (Cates, 1981), a strategy which is certainly appropriate in view of the qualities discussed above, in a habitat which can be especially exploited because of

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the small size of the insects. Moreover, rapid development times make utilisation of such short-lived resources possible.

But how do insects recognise these high sources of protein? Proteins do not appear to be phagostimulants themselves, and while a number of amino acids stimulate feeding in many instances, their concentrations are not necessarily related to the levels of overall available nitrogen. Are there other internal leaf constituents which could signal protein availability? Once again, we don't know. Thus sensory cues which can influence acceptability in such a way as to maximise protein intake are not known. They may exist, perhaps as levels of secondary compounds, but there may also be other mechanisms. There is evidence for example that nutrient feedback mechanisms controlling quantities of food eaten, could lead to restlessness on foods which are inadequate. This would then lead to effective trapping by inactivity in the vicinity of appropriate foods (Bernays & Simpson, 1982).

In conclusion, while the importance of protein cannot be overstated, the understanding of exactly how the insect herbivore is able to detect and then select the protein-rich food, or otherwise maximise ingestion of it is still rudimentary. Behaviour, and the physiological basis of the behaviour of the insect on the plant needs a much closer look, and the closer look must keep in mind the essentially insectan features, some of which have been presented here.

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Host plant preferences in larvae of the eastern spruce budworm, Choristoneura fumiferana

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Abstract

The eastern spruce budworm is a serious pest of North American forests. It feeds on new foliage from balsam fir as well as from white, red and black spruces. Sugars/glycosides from all four hosts are highly stimulating to the larva. Amino acids/bases are only slightly stimulating while organic acids are neutral or deterrent. Chloroform fractions from white and red spruces are more stimulating than those from balsam fir and black spruce. This fraction from white spruce shows the highest degree of stimulation.

Key-words: Choristoneura fumiferana, food preferences, feeding stimulants, fractionation, extraction

The eastern spruce budworm, *Choristoneura fumiferana* Clem. (Lepidoptera: Tortricidae), is a serious pest of the spruce-fir forest complex in eastern North America. The predominant host species are balsam fir *Abies balsamea*, white spruce *Picea glauca*, red spruce *Picea rubens* and black spruce *Picea mariana*. Phenologically, the spruce budworm's life cycle is attuned to the development of balsam fir and white spruce. Budburst of these two hosts coincides with the emergence of the second instar larva from its winter diapause in early May. Budburst of the other two species occurs ten days later (Swaine et al., 1924), which may account for their lowered infestation by early instar larvae.

The sensory equipment of the budworm larva is similar to that of other lepidopterous defoliators (Albert, 1980), although it has not been studied electrophysiologically to any extent. Feeding preferences of the larvae were first studied by Heron (1965), and more recently by Albert & Jerrett (1981) and Albert et al. (1981). Water-soluble components of balsam fir foliage, and especially the sugar/glycoside fraction were found to be highly stimulating to sixth-instar larvae (Albert & Jerrett, 1981). Sucrose was the most stimulating of twelve carbohydrates tested, with a peak preference in the range of 0.01 to 0.05 mol/l, which corresponds closely to the concentrations at which it occurs in balsam fir foliage (0.037 mol/l) at a time when these larvae are actively feeding in the field (Albert et al., 1981).

The present paper describes the larval feeding preferences for chemical fractions from the four host species.

Materials and methods

Foliage from all four hosts was collected at a time when larvae in the field were actively feeding as fifth and sixth instars (June 17−19, 1980) from the Acadia Forest Experiment Station, New Brunswick, Canada. Branches were collected from the mid-crown level and the current year's growth was removed and kept in sealed plastic bags over dry ice, then stored at −18°C prior to freeze-drying. Freeze-dried samples were extracted with methanol followed by column chromatography on Amberlite resins (Albert & Jerrett, 1981). The following four fractions were recovered from each host species: chloroform (lipids, organic esters, alcohols, aldehydes, waxes, etc.); sugars/glycosides; amino acids/bases; organic acids. Fractions were tested individually at concentrations of 0.1 X, 1.0 X, and 10 X; where 1.0 X was equivalent to the concentration found in the host plant at the time of collection.

Two-choice feeding tests were modified from Jermy et al. (1968) using the methodology developed for budworms by Albert et al. (1981). Test results were analysed using Wilcoxon's Signed-Ranks test (Sokal & Rohlf, 1969).

Results and discussion

A list of the materials extracted from each of the four host plant species is presented in Table 1. Sugars/glycosides accounted for the largest proportion by weight of materials recovered from balsam fir and white spruce. Balsam fir shoots at the time of collection were about 5 cm long; those of white spruce were 8 cm long. The shorter, 1.0 to 1.5 cm shoots of red and black spruces contained sugars/glycosides in weights similar to those for balsam fir and white spruce, but their organic acid contents were high compared to those of the latter two species (Table 1). Levels of amino acids/bases were similar for all four host species. Feeding preferences were tested on each extract from each plant species (Tables 2−5): balsam fir extracts of sugars/glycosides were the most preferred at the 0.1 X and 1.0 X concentrations

Table 1. Weight of chemicals (in mg/g of freeze-dried needles) recovered in each fraction from four host plants' current year's growth.

Chemical	Balsam fir	White spruce	Red spruce	Black spruce
Chloroform fraction (CH)	62.87 (20.2)[1]	75.20 (25.6)	60.26 (15.6)	99.21 (21.8)
Sugars and glycosides (SG)	178.99 (57.6)	165.65 (56.3)	155.17 (40.0)	176.51 (38.7)
Amino acids and bases (AA)	8.69 (2.8)	5.74 (1.9)	6.01 (1.6)	6.83 (1.5)
Organic acids (OA)	60.15 (19.4)	47.53 (16.2)	165.98 (42.8)	173.03 (38.0)
Total	310.70 (100)	294.12 (100)	387.42 (100)	455.58 (100)

1. Numbers in parentheses represent the % of the total weight of chemicals recovered for each host species.

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Table 2. Percent of total consumption for control disks and test disks treated with balsam fir extracts.

Control disks (H$_2$O) % ± S.E.	Test disks			n	α
	extract[1]	conc.[2]	% ± S.E.		
14.8 ± 2.1	SG	0.1 X	85.2 ± 2.1	20	0
22.8 ± 1.6	SG	1.0 X	77.2 ± 1.6	16	0
43.4 ± 2.2	SG	10.0 X	56.6 ± 2.2	23	0.007
49.6 ± 4.1	AA	0.1 X	50.4 ± 4.1	18	0.906 (ns)
36.5 ± 3.9	AA	1.0 X	63.5 ± 3.9	18	0.006
39.3 ± 4.7	AA	10.0 X	60.7 ± 4.7	21	0.046
63.4 ± 3.0	OA	0.1 X	36.6 ± 3.0	20	0
63.5 ± 4.0	OA	1.0 X	36.5 ± 4.0	19	0.008
78.6 ± 2.2	OA	10.0 X	21.4 ± 2.2	17	0
23.8 ± 3.8	CH	0.1 X	76.2 ± 3.8	18	0
54.8 ± 5.8	CH	1.0 X	45.2 ± 5.8	27	0.428 (ns)
82.9 ± 3.7	CH	10.0 X	17.1 ± 3.7	11	0.003

1. SG, sugars and glycosides; AA, amino acids and bases; OA, organic acids; CH, chloroform fraction.
2. 1 X is the concentration found in the host plant at the time of collection.
n: number of experimental animals.
α: Significance of differences in feeding between test and control.
ns: not significant.

Table 3. Percent of total consumption for control disks and test disks treated with white spruce extracts[1].

Control disks (H$_2$O) % ± S.E.	Test disks			n	α
	extract	conc.	% ± S.E.		
17.3 ± 2.8	SG	0.1 X	82.7 ± 2.8	16	0
26.4 ± 2.0	SG	1.0 X	73.6 ± 2.0	16	0
50.1 ± 2.3	SG	10.0 X	49.9 ± 2.3	24	0.988 (ns)
43.4 ± 2.5	AA	0.1 X	56.6 ± 2.5	15	0.016
29.5 ± 3.7	AA	1.0 X	70.5 ± 3.7	18	0.001
25.2 ± 3.4	AA	10.0 X	74.8 ± 3.4	18	0
59.1 ± 5.8	OA	0.1 X	40.9 ± 5.8	15	0.140 (ns)
56.9 ± 3.6	OA	1.0 X	43.1 ± 3.6	17	0.049
69.6 ± 2.2	OA	10.0 X	30.4 ± 2.2	19	0
13.6 ± 3.4	CH	0.1 X	86.4 ± 3.4	19	0
9.2 ± 2.7	CH	1.0 X	90.8 ± 2.7	20	0
20.3 ± 3.3	CH	10.0 X	79.7 ± 3.3	19	0

1. See Table 2 for legends.

Table 4. Percent of total consumption for control disks and test disks treated with red spruce extracts[1].

Control disks (H₂O) % ± S.E.	Test disks			n	α
	extract	conc.	% ± S.E.		
30.3 ± 3.3	SG	0.1 X	69.7 ± 3.3	19	0.001
24.4 ± 3.0	SG	1.0 X	75.6 ± 3.0	16	0
34.0 ± 2.8	SG	10.0 X	66.0 ± 2.8	20	0
35.0 ± 4.4	AA	0.1 X	65.0 ± 4.4	16	0.011
45.4 ± 1.9	AA	1.0 X	54.6 ± 1.9	18	0.037
40.6 ± 3.8	AA	10.0 X	59.4 ± 3.8	19	0.024
49.9 ± 2.3	OA	0.1 X	50.1 ± 2.3	12	0.859 (ns)
62.4 ± 3.3	OA	1.0 X	37.6 ± 3.3	21	0.003
80.9 ± 3.9	OA	10.0 X	19.1 ± 3.9	15	0.001
21.6 ± 5.2	CH	0.1 X	78.4 ± 5.2	20	0.001
23.1 ± 6.2	CH	1.0 X	76.9 ± 6.2	18	0.002
37.1 ± 6.4	CH	10.0 X	62.9 ± 6.4	18	0.076 (ns)

1. See Table 2 for legends.

Table 5. Percent of total consumption for control disks and test disks treated with black spruce extracts[1].

Control disks (H₂O) % ± S.E.	Test disks			n	α
	extract	conc.	% ± S.E.		
19.7 ± 2.0	SG	0.1 X	80.3 ± 2.0	20	0
24.8 ± 2.7	SG	1.0 X	75.2 ± 2.7	15	0.001
48.5 ± 3.3	SG	10.0 X	51.5 ± 3.3	22	0.897 (ns)
36.8 ± 3.8	AA	0.1 X	63.2 ± 3.8	17	0.008
29.7 ± 3.8	AA	1.0 X	70.3 ± 3.8	16	0.002
26.7 ± 3.2	AA	10.0 X	73.3 ± 3.2	17	0.001
44.1 ± 3.5	OA	0.1 X	55.9 ± 3.5	20	0.135 (ns)
52.3 ± 4.0	OA	1.0 X	47.7 ± 4.0	13	0.754 (ns)
89.0 ± 2.3	OA	10.0 X	11.0 ± 2.3	13	0.001
15.2 ± 2.7	CH	0.1 X	84.8 ± 2.7	19	0
46.2 ± 5.1	CH	1.0 X	53.8 ± 5.1	17	0.653 (ns)
78.9 ± 7.4	CH	10.0 X	21.1 ± 7.4	12	0.019

1. See Table 2 for legends.

found in the host plant (Table 2). An increase of this concentration to 10 X resulted in a decrease of the feeding preference. Amino acids/bases were non-stimulating at 0.1 X, and slightly stimulating at 1.0 X and 10 X concentrations. Organic acids deterred feeding at all three concentrations. Lipids etc. (chloroform fraction) were stimulating at 0.1 X but non-stimulating at 1.0 X and very deterrent at 10 X.

All white spruce extracts, except for sugars/glycosides at 10 X and the organic acids at all concentrations, were stimulating (Table 3). The pattern of the responses was similar to that for balsam fir except for the chloroform fraction. In white spruce, this latter fraction was more stimulating than the sugars/glycosides at each concentration.

Red spruce extracts elicited responses more similar to those for white spruce (Table 4). Here also, the chloroform fraction was as stimulating as the sugars/glycosides.

Feeding responses to black spruce extracts were similar to those for balsam fir (Table 5). However, the black spruce organic acids deterred less than those of the latter at 0.1 X and 1.0 X, even though they represent a greater proportion of the total recovered material (42.8%) than those of balsam fir (19.4%) (Table 1).

In general, larvae most preferred the white spruce chloroform fraction, followed by sugars/glycosides fractions from all four host species. Amino acids/bases were all less stimulating, and organic acids were all deterrent except for the 0.1 X concentration from all spruces and the 1.0 X from black spruce. Chloroform fractions of white spruce were stimulating at all concentrations; those of red spruce were stimulating except for the 10 X concentration and those of balsam fir and black spruce were stimulating at 0.1 X, non-stimulating at 1.0 X, and deterrent at 10 X.

The most striking results of this study are the similarities in responses between the four host plant extracts, except for the chloroform fractions. Perhaps these represent the most promising clues in the search for the difference in preferences among the species. The white spruce chloroform fraction is presently being separated in an attempt to isolate the stimulating chemicals.

Plants were collected at a time when insects in the field were actively feeding as fifth and sixth instar larvae. Balsam fir and white spruce shoots are phenologically more advanced than those of red and black spruces. The growth of the latter two species usually lags behind the first two by about ten days (Swaine et al., 1924). Nevertheless, although the host species differ in phenological age, they all stimulate feeding by larvae of the same age, and are capable of supporting larval development as indicated by the insects found on some shoots at the time of collection. Whether older shoots differ chemically from the younger shoots of the same species and whether feeding preferences for their extracted components differ with phenological age, should next be determined.

As an oligophagous insect, the spruce budworm can survive by assimilating nutrients from a range of related host species. It obviously responds differently to certain fractions of these different host plants. This raises the question of the physiological basis for this differential behaviour; more specifically, what is the sensory input to the brain that results in this differential preference for the various fractions, and directs feeding on each host? To investigate these aspects, electrophysiological studies of the chemosensilla have been initiated.

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The effect of repeated exposure to feeding deterrents on their acceptability to phytophagous insects

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Abstract

In laboratory experiments last instar larvae of two oligophagous insects (*Locusta migratoria* and *Pieris brassicae*) and two polyphagous ones (*Schistocerca gregaria* and *Mamestra brassicae*) were given food treated with secondary plant substances for a limited period each day. The concentrations used were, in each case, such as to partially inhibit feeding. In several cases the experienced larvae showed gradually increasing acceptance of feeding deterrents compared with 'naive' larvae. This phenomenon has been regarded by the authors as habituation. The increased acceptance was not related to an overall augmentation of food intake. There were striking individual differences among the larvae concerning the extent of the habituation and with *S. gregaria* aversion learning has been observed. It may be that both phenomena are interrelated and occupy the extreme ends of the same response continuum. There is some indication that polyphagous species are more likely to habituate under the given experimental conditions.

Key-words: antifeedants, *Locusta migratoria*, *Pieris brassicae*, *Schistocerca gregaria*, *Mamestra brassicae*, habituation, aversion learning

Repeated exposure to deterrents has resulted in their increased acceptability to some insects (Strebel, 1928; Gill, 1972), but data are few and the present work describes the first experiments on how various oligo- and polyphagous insects react to such exposure. This is important in understanding natural strategies of food selection and their evolutionary changes, as well as the possibilities of either using resistant cultivars whose resistance is based on non-preference, or spraying antifeedants for pest regulation.

Materials and methods

Insect species used were final larval instars of the polyphagous *Schistocerca gregaria* Forskål (Orthoptera) and *Mamestra brassicae* L. (Lepidoptera), and the oligophagous *Locusta migratoria* L. (Orthoptera) and *Pieris brassicae* L. (Lepidoptera). All were reared in the laboratory: *M. brassicae* on a semisynthetic diet (Nagy, 1970), *P. brassicae* on cabbage, and the acridids on a mixture of wheat and hay.

The deterrent chemicals were used in concentrations which caused inhibition of feeding by about 80% in caterpillars one day after ecdysis in a 17-hour non-choice test, or 90-100% inhibition in the acridids three days after ecdysis in a 2-hour non-choice test. For the caterpillars, the deterrents were mixed into the semisynthetic diets, while for acridids they were applied to the surface of sorghum leaves or added to glass-fibre paper impregnated with sucrose solution (concentration 50 g/l). All these foods are called 'deterrent diet' (DD).

In all cases a preparatory experiment was carried out. This aimed to determine the amount consumed from the DD when the larvae were exposed to it for 17 h (caterpillars) or 19 h (acridids) daily over the instar. Newly ecdysed larvae were given the 'basic diet' (BD) for the first day. This consisted of untreated semisynthetic diet for the caterpillars or wheat for the acridids. At 16 h 00 or 15 h 00 of the same day 20 individual caterpillars or 10 acridids were transferred to DD. Next day at 09 h 00 or 10 h 00 the larvae were again given BD. In the afternoon a new cycle began, and was repeated until the larvae finished feeding at the time of the next moult. The quantity of food eaten daily through a larval instar varies and the experiment was necessary to establish what quantities of food to give stock or control treatments in the main experiment when experienced insects were eating DD.

The main experiment was carried out with insects experiencing the DD day after Day 1 as in the preparatory experiment. These are the 'experienced' (E) insects. A large number of other insects of the same age, stock (S), were fed daily with measured amounts of basic diet (BD-M), limited to the quantities appropriate for their age as determined by the preparatory experiment. For the remaining hours they received BD ad libitum. Each day a new sample of these received the DD over the same period as the experienced insects. These are the 'naive' (N) insects. Quantities of food ingested were measured. Further, for the caterpillars, control larvae having only BD-M over the period when E- and N-insects had DD were set up and sampled daily (Figure 1). The DD period was 19 h for acridids and 17 h for caterpillars. Also, for acridids the BD-M was different from the BD of wheat. It was either the sorghum leaf or sucrose-impregnated glass fibre paper. Also, the N-insects were, after their single test on DD, treated as stock insects with measured amounts of BD given during the DD period, up until ecdysis. The mean amounts of BD ingested from all stock plus returned insects gave a measure of overall BD ingested when individuals had had only one exposure to DD. Thus for acridids, the naive and control treatments are compounded.

Results

Increased intake of deterrent food   In both experiments with *M. brassicae* larvae the daily intake of deterrent diet by the experienced (E) larvae increased relative to the naive (N) larvae, so that the total DD consumption of the two groups of larvae also differed significantly (Figure 2). The same applies to the strychnine experiment with *P. brassicae*. However, while the intake of DD increased in *M. brassicae* gradually during the instar, it only appeared in *P. brassicae* towards the end of the feeding period. In these three experiments with caterpillars total food consumption of experienced (E) larvae and control (C) larvae was the same, but naive larvae consumed

DAY 1

Dawn: Moulting of larvae and feeding on BD <u>ad lib.</u>

14.00   Weighing of larvae, randomizing

16.00   S   15 C   15 N   19 E
            BD-M   BD-M   DD   DD

DAY 2

09.00   BD <u>ad lib.</u>   BD   BD   BD

15.00   discard discard

16.00   15 C   15 N   weigh
               weigh weigh
         BD-M   BD-M   DD   DD

DAY 3
etc.
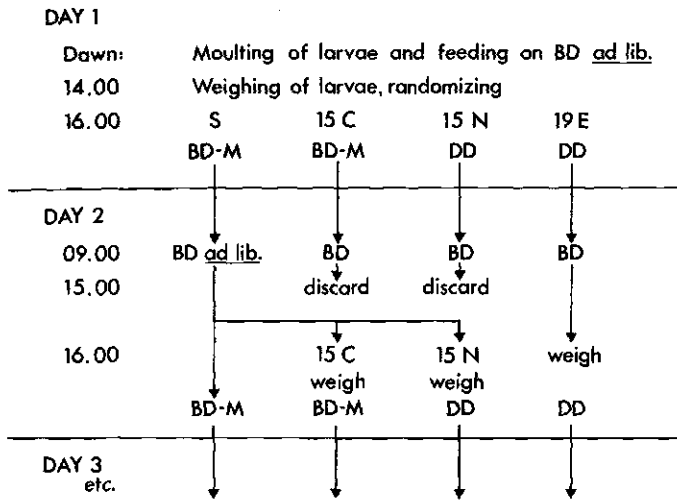
Fig. 1. Design of main experiment with caterpillars. S: stock; C: control; N: naive; and E: experienced insects. BD: basic diet; BD-M: basic diet in limited, measured amount; BD ad lib.: basic diet ad libitum; DD: deterrent diet. All food intake was monitored.

significantly less food than the other two groups. In the quinine experiment with *P. brassicae*, the control larvae consumed least (Table 1).

In both lepidopterous species, and particularly in *P. brassicae*, the experienced larvae showed striking individual differences in their ability to increase consumption of deterrent diet.

Increased ingestion of the deterrent diet was found also in *S. gregaria* when nicotine hydrogen tartrate (NHT) was applied to the surface of sorghum leaves (mass

Table 1. Total food intake (mg dry matter ± SD) of caterpillars with different deterrents and treatments.

Larval group[1]	*Mamestra brassicae*		*Pieris brassicae*	
	strychnine 0.13 mol/l	quinine 0.1 mol/l	strychnine 2.2×10^{-4} mol/l	quinine 2×10^{-4} mol/l
E-larvae	575 ± 69 a[2]	463 ± 82 a	353 ± 73 a	381 ± 49 a
(DD + BD)	(19)[3]	(17)	(13)	(19)
N-larvae	383 ± 51 b	299 ± 53 b	221 ± 29 b	334 ± 65ab
(DD + BD)	(15)	(15)	(15)	(11)
C-larvae	516 ± 34 a	511 ± 60 a	318 ± 24 a	325 ± 37 b
(BD + BD-M)	(15)	(15)	(15)	(11)

1. E: experienced; N: naive; C: control; DD: deterrent diet; BD: basic diet; BD-M: basic diet in limited, measured amount.
2. Different letters in the same column indicate significant differences at $P = 0.001$.
3. Number of replicates.

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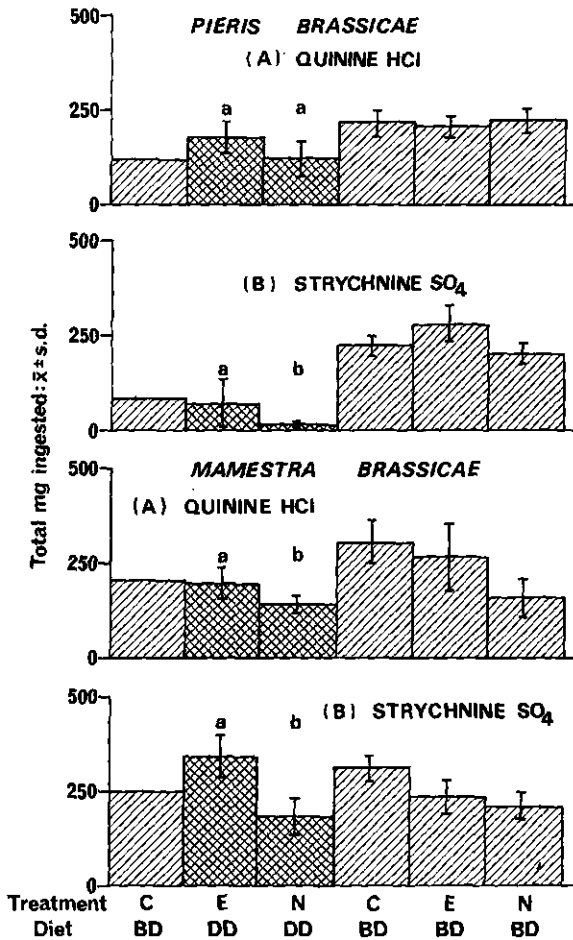
Fig. 2. Average amounts of basic and deterrent diets, respectively, consumed per larva during the whole feeding period (dry matter). For the meaning of the letters for treatment and diet see legend Figure 1. Different letters above columns indicate significant differences at $P =$ 0.001. For numbers of larvae see Table 1. Note that BD was given 7 h/day (right three columns) and other diets 17 h/day (left three columns).

fraction in dry matter approximately 9 g/kg). The difference was significant for most days of the instar, with a considerable overall difference in consumption of deterrent diet but not basic diet (Figures 3 and 4).

An increase in ingestion of deterrent diet over the instar by experienced larvae of *L. migratoria* occurred when this diet was sorghum with nicotine hydrogen tartrate at mass fraction in dry matter of 3 g/kg (Figure 3). However, the increase in this case was of a quite different nature and resulted not from an obviously gradual
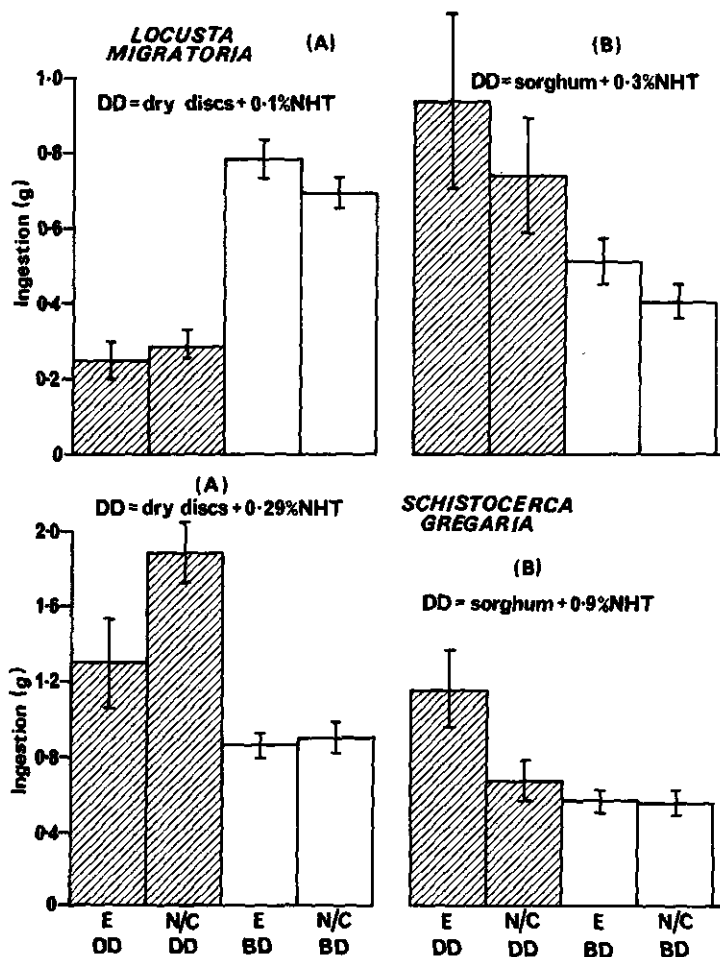
Fig. 3. Average amounts of basic and deterrent diets, respectively, consumed by acridids in four different experiments (dry matter). NHT: nicotine hydrogen tartrate. Vertical bars indicate standard deviations. For the meaning of the letters for treatment and diet see legend Figure 1. Ten insects in each treatment. Note that BD was given 5 h/day (open columns), and other diets 19 h/day (dark columns).

change, but from a prolonging of the instar through some physiological effect of the alkaloid, so that the feeding period lasted longer. Figure 4 demonstrates the contrast between increasing ingestion of deterrent diet by experienced larvae from early in the instar, and an increase in the second half of the instar associated with delayed moulting. A similar phenomenon occurred with this species when the deterrent diet was sucrose-impregnated glass fibre paper with sinigrin (mass fraction in dry matter 1 g/kg), and with S. gregaria having a diet of sucrose-impregnated glass fibre paper with quinine hydrochloride (mass fraction in dry matter 10 g/kg). There was no increase in mortality in the E-larvae of these experiments.

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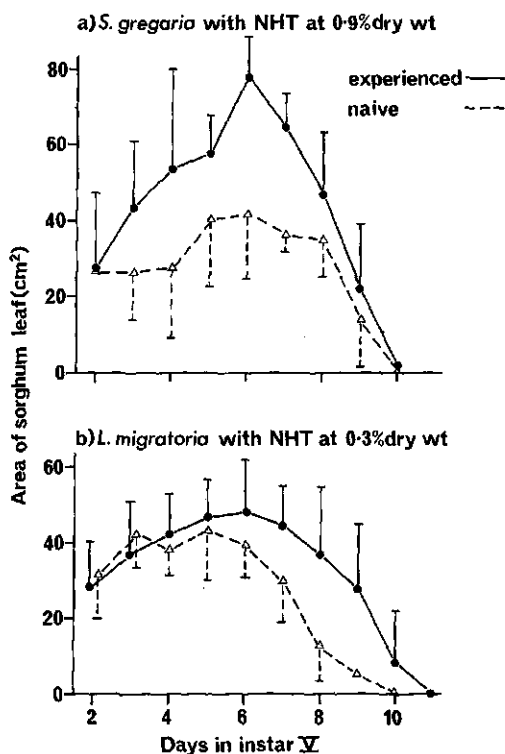
Fig. 4. Quantities of deterrent diet consumed daily over the instar by a) *Schistocerca gregaria* with nicotine hydrogen tartrate (NHT) on sorghum leaves, and b) *Locusta migratoria* with NHT on sorghum leaves. Vertical lines represent standard deviations.

Decreased intake of deterrent food This was only found with *S. gregaria*, and only when the diet was sucrose-impregnated glass fibre paper with added chemicals. Thus NHT on this substrate was eaten significantly less by the E-larvae than by the N-larvae, in marked contrast to the situation when the NHT was presented on leaf material (Figure 3). Similar results were obtained with azadirachtin at mass fraction in dry matter of 2.5 μg/kg, and linalool at mass fraction in dry matter of 0.2 g/kg (initial concentration).

No change of deterrent food intake This was the case with some deterrents tested with *L. migratoria* with sucrose-impregnated glass fibre paper plus test chemical as the deterrent. It was found with linalool, NHT and tannic acid at mass fractions of 0.1, 0.1, and 50 g/kg respectively. It was also shown with *P. brassicae* with quinine at substance concentration 0.2 mmol/l where diet consumption of E- and N-larvae differed, but only at a probability of 0.005, which was not regarded as sufficient under such experimental conditions.

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Mortality caused by ingestion of deterrents This occurred in *L. migratoria* with daily ingestion of azadirachtin on glass fibre filter paper, at mass fraction in dry matter of 0.05 g/kg. Insects which ingested 5 µg or more usually died. Some individuals however, refused to eat the diet with this concentration of azadirachtin.

Discussion

The method used in all experiments aimed to minimize the differences in the degree of deprivation between the different groups of larvae by feeding the S- and C-larvae limited, measured quantities of basic diet, while the E- and N-larvae were given deterrent diet (Figure 1). This nutritional equivalence, is not a behavioural equivalence however, since the S- and C-larvae consumed the limited basic diet (BD-M) within the first few hours while the other two groups ate variably and intermittently on the deterrent diet during the whole exposure time (17 or 19 h).

In spite of this, an increased intake of deterrent diet resulted from increased exposure to it in several experiments. In three of the four experiments with caterpillars there was a significant and considerable behavioural change with no obvious physiological effects of the chemicals on them: the total food consumption of E- and C-larvae did not differ significantly (no noticeable extra cost), the final weights of the larvae were the same (no obvious deleterious effect), and there was no increase in mortality of the E-larvae. Similarly in the case of *S. gregaria* exposed to NHT on sorghum leaves, E-larvae ate considerably more of the deterrent diet with no indication of a cost in terms of extra ingestion of basic diet, time of ecdysis or final weights, which were not different in the E- and N-larvae. Since, in these experiments, the increase in tolerance to the deterrents is a consequence of the waning of the response to the inhibitory stimulus (Thompson & Spencer, 1966), it can properly be regarded as habituation. With the caterpillars, the N-larvae had a significantly reduced total food intake, as a result of a restricted feeding intensity on the basic diet for the six hours after exposure to the deterrent diet. In these cases the deterrent seemed either to invoke an inhibitory state in some neural centre coordinating food intake (Dethier et al., 1968; Jermy, 1971) or perhaps induce a general inactivity for a period. Repeated exposure apparently overcame the effect in the E-insects.

These marked increases in ingestion of deterrent diet throughout the instar are in contrast to the increased ingestion occurring at the end of the instar in some experiments in relation to a delay in ecdysis. In these cases habituation is not indicated, and presumably the costs to these insects would cause a continual decline in fitness if ingestion of the deterrent diet continued.

Decreased intake of deterrent food over a period as seen in experiments with *S. gregaria* when linalool or NHT was applied on glass fibre paper may be aversion learning, since it was not accompanied by a decrease in feeding intensity. In its appearance this response seems to be analogous to the aversion learning found by Dethier (1980) in certain caterpillars, except that in *S. gregaria* there was no noticable evidence of toxicity, which is generally believed to be associated with aversion learning. The unnatural inadequacy of the 'diet' however, is probably in some way responsible for the effect, and it must be emphasised that the same deterrent (NHT) when applied on sorghum leaves evoked habituation. It may be that both phe-

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nomena are interrelated and occupy the extreme ends of the same response continuum, the type of response to a given deterrent depending on the overall stimulus situation.

The lack of any effect of the deterrents which was found in *L. migratoria* with several chemicals tested on the glass fibre paper, as well as the fact that habituation appeared very late in *P. brassicae* with strychnine, and hardly at all with quinine, seem to indicate that oligophagous species are more rigid in their responses to deterrents.

In conclusion, the response to repeated exposure to deterrents is very variable. Although habituation has been shown, no general rules can be drawn for the understanding of food selection strategies in nature. In addition, choice situations may modify the effect. It is important to note however, that the dry inadequate diet gave results differing greatly from those obtained with the good quality leaf diet. More work is needed to investigate the different responses in relation to species or group and the overall feeding behaviour, including the type of phagism. It is also important to know whether the striking individual differences are hereditary. Progress in the practical use of antifeedants and non-preference plant resistance will depend on an understanding of these problems.

Acknowledgments

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Effect of a benzoxazinone from wheat on aphids

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Abstract

2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), the main benzoxazinone iso-lated from wheat extracts, decreases aphid survival and reproduction rates in artificial diets. The effect of the naturally-present 2-0-β-D-glucoside was less than that of DIMBOA. There-fore, hydrolysis of the glucoside upon infestation may be required for resistance of cereals to aphids. The biological activity of DIMBOA is decreased by addition of cysteine to the diets. DIMBOA reacts with thiols with rates proportional to reduction potentials of thiols. With ethanethiol, DIMBOA gives addition and/or reduction products. These properties of DIM-BOA may be related to its mode of action.

Key-words: wheat, *Metopolophium dirhodum, Schizaphis graminum, Rhopalosiphum maidis*, hydroxamic acids, DIMBOA, plant resistance, survival, reproduction

Wheat extracts contain benzoxazinones (Willard & Penner, 1976), the most abun-dant of which is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA, Figure 1), a cyclic hydroxamic acid. This and related hydroxamic acids are important in resistance of several Gramineae to insects (Klun et al., 1967; Argandoña et al., 1980, 1981). In addition DIMBOA inhibits bacterial growth in culture and spore germina-tion (Elnaghy & Linko, 1962; Corcuera et al., 1978). In this paper we describe the effects of DIMBOA on aphids fed with artificial diets and explore chemical proper-ties of DIMBOA that may be related to its mode of action.

Materials and methods

Seeds were obtained from Instituto Nacional de Investigaciones Agropecuarias, Departamento de Sanidad Vegetal, Universidad de Chile, and Sociedad Nacional de Agricultura.

Before extraction and purification of the compounds involved, the plant tissue was macerated in water and filtered through cheese-cloth. The extract was adjusted to pH 3 with aqueous HCl (1 mol/l) and centrifuged in a force field of 8500 g_n (83.3 kN) for 15 min. The supernatant was extracted three times with 2 volumes of ethyl ether and the organic phases were evaporated to dryness. These extracts were

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1a R = H   DIMBOA
1b R = Glc DIMBOA
          glucoside

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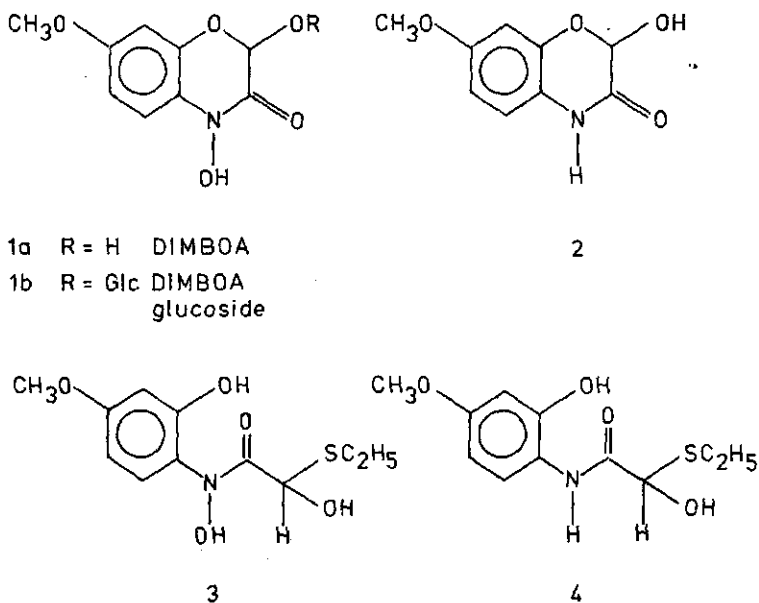
Fig. 1. Products (2,3,4) isolated from the reaction of DIMBOA (1a) with ethanethiol.

used for quantitation of hydroxamic acids, and to isolate DIMBOA by a procedure previously described (Woodward et al., 1978b). Coleoptiles of 6-day old seedlings of *Zea mays* L. cultivar LH Rinconada were used to isolate DIMBOA.

The 2-0-β-D-glucoside of DIMBOA was obtained from aqueous extracts of boiled maize seedlings which were passed through SP-Sephadex-Fe (Corbett & Chipko, 1978) and Sephadex G-10 columns (Hofman & Hofmanova, 1969). UV, IR and NMR spectra of DIMBOA and its glucoside were obtained.

Hydroxamic acids form a blue complex upon addition of $FeCl_3$ reagent (50 g of $FeCl_3.6H_2O$, 500 ml aqueous ethanol (volume fraction 95%) and 5 ml of HCl (concentration 14 mol/l)). The concentration of hydroxamic acids in the tissues was determined by comparing the absorbance of the extracts with a standard curve made with DIMBOA ($\lambda_{max} = 590$ nm, $\varepsilon_{590} = 131.5$ $m^2.mol^{-1}$). Thus, the values reported represent DIMBOA equivalents (Woodward et al., 1978a; Argandoña et al., 1980). Rates of disappearance of hydroxamic acids from solutions of DIMBOA were followed by withdrawing aliquots, adding them to $FeCl_3$ reagent and measuring the absorbance at 590 nm.

Aphids were collected from fields near Santiago and allowed to reproduce on barley plants kept inside a nylon cage in the laboratory. For feeding experiments, a pH 5.5 aqueous solution of 30% sucrose, amino acids, vitamins and mineral salts placed between two layers of Parafilm M was used (Auclair, 1965; Argandoña et al., 1980).

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Table 1. Hydroxamic acid content and susceptibility of several cultivars of wheat to *Schizaphis graminum*.

Wheat	Hydroxamic acids in leaf extracts (mmol/kg fresh weight)	Aphids/sample initial[1]	final	Population growth rate[2] (per day)
Triticum durum				
cv. SNA-1	1.85	6	21	0.21
Tricitum aestivum				
cv. Naofen	1.50	6	25	0.24
cv. Cajeme	1.37	6	32	0.36
cv. Likay	0.89	6	83	0.43
cv. Sonka	0.61	6	86	0.44

1. The infestation was carried out in 10-day old greenhouse-grown plants. The experiment lasted 6 days. Each sample consisted of 6 plants.
2. Growth rate $= (\ln n_f/n_i)/\Delta t$.

Results

Several varieties of wheat were infested with *Schizaphis graminum*. Hydroxamic acids in the leaves as well as aphid population growth rate were measured (Table 1). A negative correlation was found between hydroxamic acid content and aphid population growth rate on the same leaves, suggesting a possible role of these compounds in resistance of the plants to the *S. graminum*.

Three species of aphids which normally attack Gramineae were fed with artificial diets with or without DIMBOA (Figure 2). A major decrease in survival of *S. graminum* and *Metopolophium dirhodum* was observed while *Rhopalosiphum maidis* was not greatly affected. At lower concentrations DIMBOA decreased the reproduction rate of *S. graminum* (Figure 3).

Survival of *S. graminum* in diets was greater when aphids were fed DIMBOA-glucoside than DIMBOA (Figure 4), suggesting that the naturally-present glucoside is not the active compound.

The half-life of DIMBOA in solutions containing cysteine was shorter than in solutions without the aminoacid (Table 2). The products of the reaction of DIMBOA with cysteine were not toxic to *S. graminum* (Table 3). The toxicity of DIMBOA to *S. graminum* was decreased by the presence of cysteine in the diets (Table 3). Since most media used to assay biological activity of DIMBOA contain cysteine, it may be concluded that the activity of DIMBOA is greater than previously reported.

Products isolated from the reaction of DIMBOA with ethanethiol are shown in Figure 1, marked as 2, 3 and 4 (Niemeyer et al., 1982). They arose from hemiacetal-hemithioacetal exchange and/or from reduction of the hydroxamic acid to amide. Second-order rate constants for the reaction of DIMBOA with cysteine, mercaptoethanol and dithiothreitol were determined (Table 4). The logarithms of these rate constants were linearly correlated to the reduction potentials of the thiols (Table 4), suggesting that the rates measured corresponded to the reduction step. This is sup-

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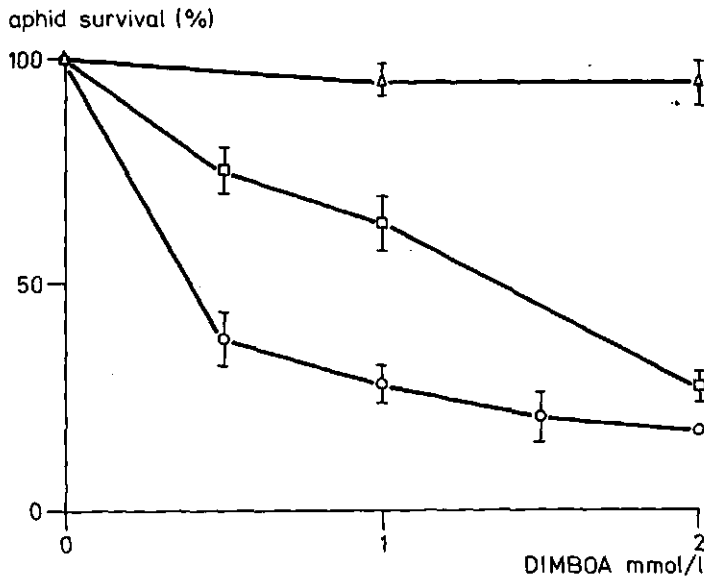
Fig. 2. Effect of DIMBOA on *Rhopalosiphum maidis* (△), *Metopolophium dirhodum* (□) and *Schizaphis graminum* (○). Survival was measured after feeding aphid nymphs with an artificial diet for 24 h. Each point is the mean of three samples of ten individuals each. Vertical lines indicate standard errors.
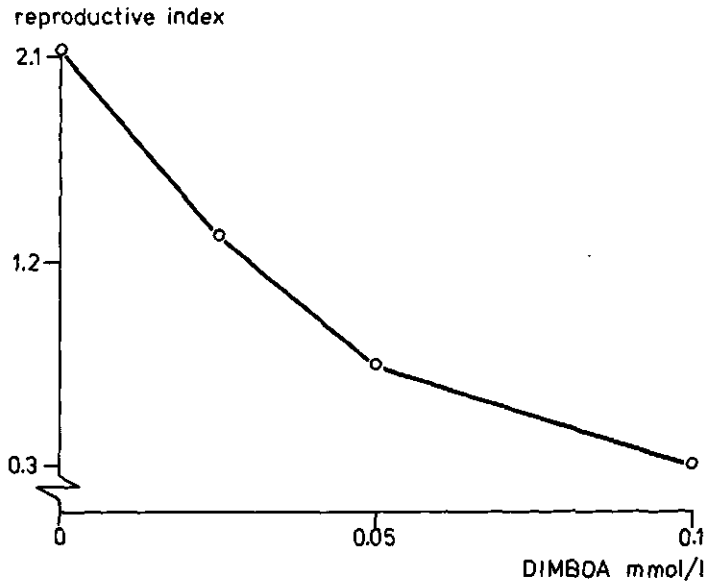
Fig. 3. Effect of DIMBOA on *Schizaphis graminum* fed with artificial diets. The reproductive index (number of nymphs/average number of adults) was measured after feeding aphid adults for 72 h.

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Table 2. Effect of cysteine on the rate of decomposition of DIMBOA at substance concentration 4 mmol/l in insect diet and in pH 5.5 potassium hydrogen phthalate at 28°C.

Solution	Cysteine (mmol/l)	Half-life of DIMBOA (h)
Buffer	0.0	50.8
Buffer	2.8	34.1
Insect diet	0.0	48.2
Insect diet	2.8	31.9

Table 3. Effect of cysteine, DIMBOA and DIMBOA decomposition products on survival of *Schizaphis graminum*.

Concentration in diet (mmol/l)		Aphid survival after 24 h (%)
cysteine	DIMBOA	
0.0	0.0	100
2.8	0.0	100
0.0	4.0 (decomposed)[1]	100
2.8	4.0 (decomposed)[1]	100
0.0	4.0	20
2.8	4.0	40

1. Prior to feeding, DIMBOA (4 mmol/l) was decomposed for a period of 14 half-lives (see Table 2) in insect diets with or without cysteine.

Table 4. Rate constants (k_2) for the reactions of DIMBOA with thiols.

Thiol	k_2 (1/mol · min)[1]	$-E_0$ (V)[2]
Dithiothreitol	0.98	0.37
Mercaptoethanol	0.73	0.28
Cysteine	0.54	0.21

1. Per thiol group in the molecule. Reactions were carried out at 31°C in pH 8 phosphate buffer.
2. Reduction potential.

ported by rate data on the addition of water (Cliffe & Waley, 1961) and of thiols (Lienhard & Jencks, 1966) to aldehydes. These are substantially faster than our measured rates.
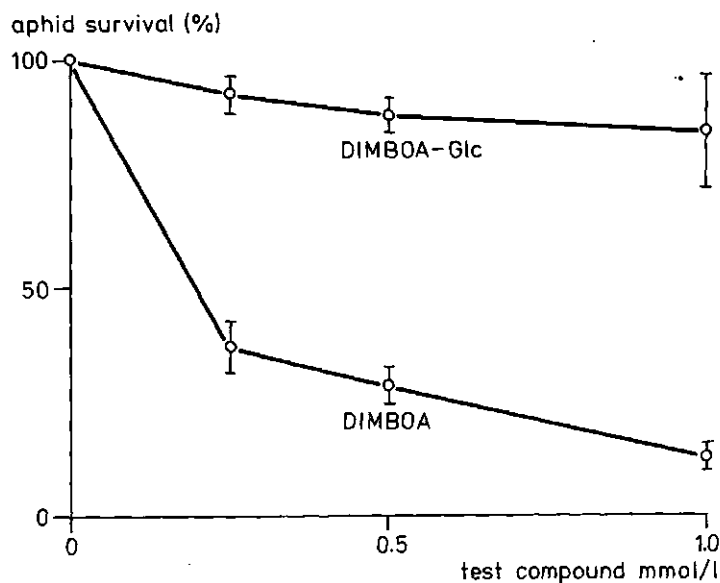
Fig. 4. Effect of DIMBOA and DIMBOA-glucoside on *Schizaphis graminum* fed with artificial diets. Survival was measured after feeding the aphids for 48 h. Each point is the mean of three samples consisting of ten aphids each. Vertical lines indicate standard errors.

Discussion

DIMBOA decreased survival and reproduction rate of *S. graminum* fed with artificial diets. The content of this compound in leaves correlated with resistance to the aphid. It is likely then that these hydroxamic acids constitute a chemical defense of wheat against these aphids. The effects in artificial diets were observed using similar or lower concentrations of hydroxamic acids than those found in plant extracts.

The mode of action of these compounds on aphids is presently unknown. Hydroxamic acids could affect aphids by decreasing reproduction, increasing mortality and/or acting as feeding deterrents.

The reactivity of DIMBOA with thiols suggests that DIMBOA may be an inhibitor of enzymes whose activity depends on the presence of cysteine residues. DIMBOA has been described as an energy transfer inhibitor in chloroplasts and mitochondria (Queirolo et al., 1981). Although it is not known how DIMBOA decreases ATP synthesis in these organelles, it is possible that its reactivity with cysteine is responsible for this action.

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Roles of mixed-function oxidases in insect herbivory

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Abstract

The gut microsomal mixed-function oxidase (MFO) system is implicated in protecting herbivorous insects from defensive plant allelochemics. This paper evaluates this concept and shows that: (1) in Lepidoptera, e.g., the gypsy moth, *Lymantria dispar*, the MFO titer increases both ontogenetically and by induction, suggesting an increase in biochemical defense commensurate with larval development and concomitant increase in food consumption; (2) the MFO system in Coleoptera, e.g., the Japanese beetle, *Popillia japonica* also appears to respond to allelochemics of host plants in a manner similar to lepidopterous larvae; (3) the increase in MFO level during feeding stages, and a decline during quiescent developmental stages, markedly affects insect susceptibility to insecticidal compounds that also are MFO substrates; (4) high levels of MFO enzymes present in insect fat bodies may serve as a secondary line of defense against allelochemics; and (5) the gut MFO system also may have functions other than allelochemic detoxication.

Key-words: detoxication, *Lymantria dispar*, mixed-function oxidases, secondary plant metabolites, gut, fat bodies, ontogenesis.

The microsomal mixed-function oxidase (MFO) system is well-known for its role in primary degradation and deactivation of lipophilic drugs, pesticides, and other synthetic compounds. It functions as an electron transport system whose terminal enzyme is cytochrome P-450, and the reducing equivalents for the system are provided by NADPH. Bioassay of microsomal preparations of insect tissues obtained by two-step high-speed spins of crude homogenates (Figure 1) for MFO activity may use various model substrates, e.g., aldrin, *p*-chloro-*N*-methylaniline, and methoxyresorufin (Brattsten, 1979), or may follow overall oxidation of NADPH to NADP+ (Ahmad & Forgash, 1973).

In lepidopterous larvae, particularly polyphagous species, the MFO system has the attributes of an effective biochemical defense against dietary stress of plant allelochemics (Krieger et al., 1971; Brattsten et al., 1977). Similar results have been obtained very recently for the MFO system of a highly polyphagous coleopteran, the Japanese beetle, *Popillia japonica* Newman (Ahmad, 1982). However an investigation of the two-spotted spider mite, *Tetranychus urticae* Koch, by Gould et al. (1982) has questioned the role of the MFO system as a survival mechanism against

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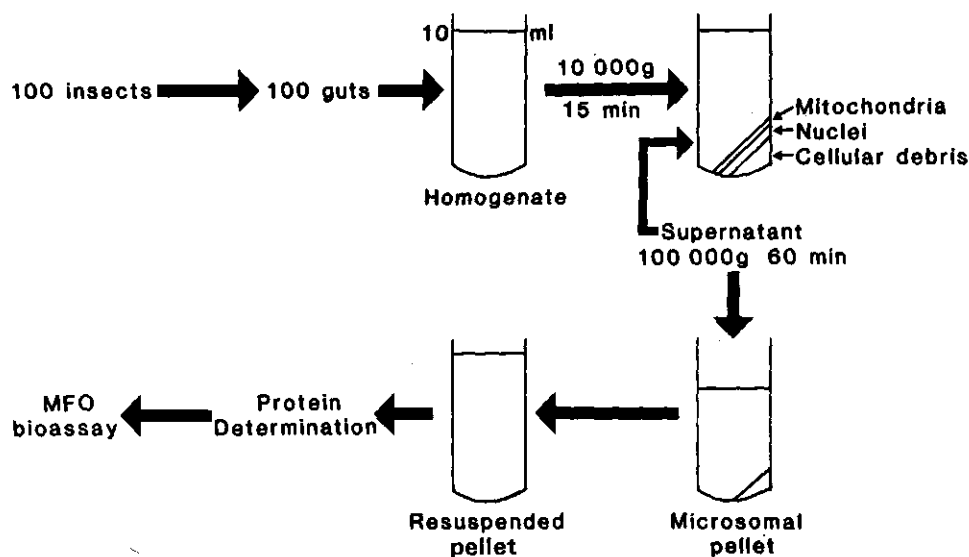
Fig. 1. Procedure generally used in obtaining insect gut microsomes. Microsomes from other insect tissues are similarly obtained. Following preparation of the microsomal suspension, the protein content (mg/ml) is first determined, and then MFO assay conducted by one of several methods (see text).

allelochemics. This paper evaluates the function of the MFO system in insect herbivory in light of recent publications and some unpublished work.

Strategic distribution of MFO activity in insect tissues

In insects MFO activity occurs in the gut, fat body, and Malpighian tubules. Gut activity is generally higher than the other two tissues in herbivorous insects, e.g., in the southern armyworm, *Spodoptera eridania* (Cramer) (Krieger & Wilkinson, 1969); gypsy moth, *Lymantria dispar* L. (Ahmad & Forgash, 1973); saturniid moth, *Antherea pernyi* (Guerin) (Krieger et al., 1976); black cutworm, *Agrotis ypsilon* (Hufnagel) (Thongsinthusak & Krieger, 1976); house cricket, *Acheta domesticus* L. (Benke & Wilkinson, 1971); and the roach, *Gromphadorhina portentosa* (Schaum) (Benke et al., 1972). The gut is the first organ to encounter dietary allelochemics, so high MFO activity in this tissue is essential to effective detoxication of toxic allelochemics before they enter the hemolymph.

Furthermore, Krieger et al. (1971) showed that the guts of generalist lepidopterous larvae have significantly higher MFO titers than specialist species. This is consistent with the assumption that polyphages are subject to exposure to a wider range, and possibly also greater amounts, of allelochemics in their numerous hosts. A subsequent investigation supporting this finding revealed high gut MFO titers in the adults and larvae of the highly polyphagous Japanese beetle (Ahmad, 1982).

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Implications of ontogenetic changes and dietary induction of gut MFO

In insects, MFO activity can increase or decrease depending upon the developmental phase and feeding activity. For example, in the gypsy moth, the activity declines during each larval moult cycle (non-feeding period), but the basal titer (immediately after ecdysis) of the subsequent instar is higher than the preceding stage. The MFO activity of non-feeding developmental stages is low compared to actively feeding larval or adult stages. For example, in the Japanese beetle, the prepupae and pupae have only 40% of the MFO specific activity of third-instar larvae (Ahmad, 1982).

MFO activity in feeding stages also is subject to induction by MFO substrates present in the food plant. Figure 2 shows the combined effects of ontogenetic changes and induction in the gypsy moth larva. Differences in MFO titres of different instars immediately after ecdysis are due to ontogenetic changes, but the increase in MFO levels during an instar (i.e., early to mid instar) represents induction. The enzyme levels for pin-oak-fed larvae are different from wheat germ-reared larvae, presumably because of differences in potential inducers, and also differences in the consumption of the two diets; the data nevertheless clearly show a close relationship between MFO titer and increase in food consumption concomitant with larval advancement.

More refined studies have shown a rapid response of the MFO system to the presence of allelochemics in the food. Within 24 h of feeding on a diet containing (+) α-pinene, gut MFO activity increases several fold (Brattsten et al., 1977); with this induction, the armyworms attained higher tolerance to the toxic alkaloid, nicotine. The gut MFO titre of the Japanese beetle adult is also induced maximally within 24 h of feeding on common sassafras (*Sassafras albidum*), perennial phlox (*Phlox paniculata*), or broccolli (*Brassica oleracea botrytis*) (Ahmad, 1982). Moreover, MFO level was about 2.5 times higher after laboratory feeding of the beetles on the three plants together than on single host species. The beetle larvae, like the adults, also are polyphagous and feed on roots of a great variety of grasses, legumes, cultivated crops, weeds, and shrubs. However, they often occur as facultative monophages, for example, feeding on roots under a homogeneous turfgrass patch. Bioassays of the larval gut MFO system showed that the enzyme level was significantly higher after feeding on a mixture of annual bluegrass (*Poa annua*), Diplomat perennial ryegrass (*Lolium perenne*), and Banner chewing fescue (*Festuca rubra*) than after feeding on bluegrass alone (Ahmad, 1982). Taken together these data support the earlier conclusions of Krieger et al. (1971) and Brattsten et al. (1977), that the MFO system is highly flexible and responds to feeding habits and strategy of phytophagous insects.

Correlation of MFO activity to insecticide susceptibility

Changes in MFO activity with insect development affect insecticide susceptibility of the house cricket (Benke & Wilkinson, 1971), *G. portentosa* (Benke et al., 1972), and the black blowfly, *Phormia regina* (Meigen) (Wilkinson & Brattsten, 1972). Changes in the microsomal MFO activity also is correlated to in vivo toxicity of the insecticides, carbaryl and diazinon (Ahmad & Forgash, 1975). Non-feeding late

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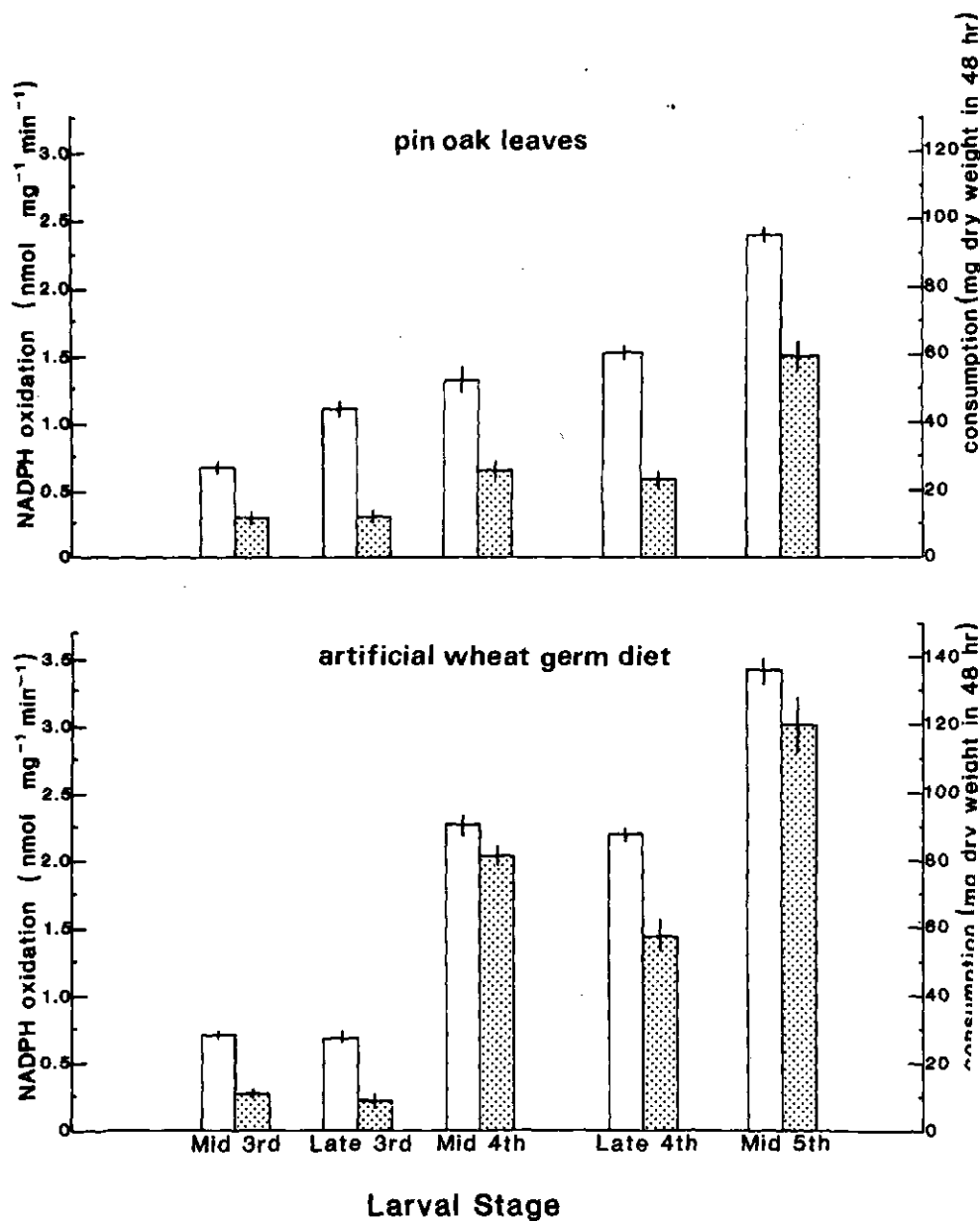
Fig. 2. Relationship between larval food consumption (dotted bars) and gut MFO activity (open bars; measured as overall NADPH oxidation) of gypsy moth larvae. Data on NADPH oxidation are from Ahmad & Forgash (1978). Preweighed fresh leaves or diet blocks were provided to a batch of ten larvae. Mean diet consumption over 48 h was obtained from eight such replicates. The ratios of wet to dry mass of leaves and diet blocks were determined by weighing samples before and after drying at 90°C for 6 h. The quantity of food consumed was converted to a dry mass basis using these ratios. Vertical lines indicate standard errors.

third-instar larvae of the Japanese beetle are 18 and 37 times more susceptible to latent toxicity of bendiocarb and chlorpyrifos, respectively, than are actively feeding early third-instar larvae (Ng & Ahmad, 1980). We hypothesized that with the maturation of the instars into non-feeding late third-instars, MFO activity declines, allowing small amounts of insecticides to persist long enough in intact form to produce latent toxic effects. Subsequent measurements of the larval MFO activity showed a drastic drop (by as much as 60%) as the larvae matured into late third-instar larvae (Ahmad, 1982).

MFO induction by plant allelochemics raises insecticide tolerance, as shown for variegated cutworm, *Peridroma saucia* (Hubner), reared on peppermint (Yu et al., 1979), and armyworms' tolerance to nicotine following MFO induction by (+) α-pinene (Brattsten et al., 1977). However, although spider mites selected for survival on a toxic host (a resistant cucumber variety) had higher resistance to organophosphorous insecticides than the control population, insecticide-resistant population did not survive better on toxic cucumber (Gould et al., 1982). The lack of the anticipated correlation remains unexplained but may be due to multiplicity of cytochrome P-450's of the MFO system. Qualitative and quantitative differences in cytochrome P-450 have been discerned on the basis of differential effects of inhibitors on various types of reactions catalysed by a single microsomal preparation, by unequal degrees of MFO induction, and by different forms of cytochrome P-450 present in a single species (Ahmad, 1979). Herbivores reared on a food plant are exposed to many different allelochemics; their multiple cytochromes are therefore likely to be induced simultaneously. One or more of the induced forms may be efficient in detoxifying insecticides, e.g., organophosphorous compounds. On the other hand, the exposure to one class of compounds, i.e., organophosphorous compounds, is likely to induce only a specific form of cytochrome P-450, which may not confer a broad tolerance to a host of different allelochemics present in one food plant.

A possible role for microsomal fat body MFO system

Microsomal MFO activity is present in the fat body of all insect species investigated. In the Japanese beetle, the specific activity of fat body MFO is as much as 85% that of gut (Ahmad, 1982), and in some species, e.g., the cabbage looper, *Trichoplusia ni* (Hubner), the activity surpasses that in the gut (Kuhr, 1970). The exact function of the fat body's microsomal MFO system remains largely unexplored. Also in the omnivorous cockroach, *Periplaneta americana* L., MFO activity is highest in fat body microsomes. The cockroach fat body is an amorphous mass of tissue in close contact with both the integument and the gut. Therefore, Wilkinson & Brattsten (1972) suggested that the fat body MFO could provide roaches protection from integumental entry of toxicants. This hypothesis is quite plausible, but toxins (e.g., modern synthetic toxicants) absorbed via the integument probably became important only very recently in the evolutionary history of the cockroach. It is conceivable that the original selective advantage for the elaboration of fat body MFO was to provide insects with a secondary line of defense from toxic food-plant allelochemics. The work on armyworm's (Brattsten, 1977) and Japanese beetle's gut MFO has clearly shown that there is an upper limit for the induction (about 4-fold).

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Therefore, during a prolonged feeding episode generalist insects may sometime ingest more toxic allelochemics than can be efficiently detoxified by the gut MFO system, and some of these may escape into hemolymph unmodified. The fat body bathing freely in the hemolymph could, with its MFO system, act as a second line of defense against toxic allelochemics in the hemolymph. This hypothesis awaits experimental investigation.

Other possible functions of gut microsomal MFO system

Many types of biosyntheses occur in insect gut, including conversion of plant sterols to cholesterol. The phytosterols are typically C_{28} and C_{29} steroids, which undergo a series of metabolic transformations, leading to the production of C_{27} cholesterol. One of these critical transformations may be epoxidation, which is typically catalysed by the MFO system (Ahmad, 1979). Therefore, the participation of the gut MFO system in the synthesis of cholesterol is a strong possibility that awaits experimental support (Ahmad, 1979). Insects also exploit host plant substances for the synthesis of sex attractants, and the MFO system might participate in the synthesis of pheromone molecules, e.g., synthesis of disparlure from plant olefins, and heterocyclic pheromones of danaid butterflies from the toxic pyrrolizidine alkaloid, monocrotaline (Brattsten, 1979).

Given the wide range of substrates, and multiplicity of the cytochrome P-450 of the MFO system, it is quite likely that more endogenous roles for the MFO system await discovery. The MFO system involved in biosynthetic conversions may be distinct from the more general detoxication system with which it may coexist in different compartments in the endoplasmic reticula of the gut, or the difference may be in the cytochrome P-450. Clearly the diversity of cytochrome P-450, its substrate specificity, and reactions catalysed, need immediate attention to unravel the biochemical basis of a multitude of possible reactions of the insect MFO system on plant allelochemics.

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Conditions of feeding preference for wilted sunflower in the grasshopper Melanoplus differentialis

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Abstract

The grasshopper *Melanoplus differentialis* prefers wild and cultivated sunflower leaves wilted by girdling beetles or by excision to turgid leaves. All instars and both sexes of adults exhibit the preference for excised leaves in laboratory choice tests, unless food- en water-deprived for 24 h. Leaves with a water loss of 50 g/kg are preferred to turgid leaves. Dried leaves and ones wilted and rehydrated to full water content are also preferred. Grasshoppers given a choice for 24 h between a turgid leaf and a dried one eat significantly less from the turgid leaf when also provided with free water than ones given no free water. No preference is shown when test leaves are taken from wild plants characterized as less vigourous or unfertilized cultivated plants. These and other results suggest that the preference is due to a non-water chemical.

Key-words: Helianthus annuus, plant stress, plant vigour, *Melanoplus differentialis*, food preferences

Field observations in central Texas, USA, revealed that the polyphagous grasshopper *Melanoplus differentialis* (Thomas) (Orthoptera: Acrididae) prefers sunflower, *Helianthus annuus* L., leaf tissue that is infected with rust fungus, damaged by leaf feeding insects, or wilted by girdling beetles, to adjacent undamaged leaves (Lewis, 1979, 1981). Additional field and laboratory observation confirmed similar preferences by this grasshopper on 17 of 21 host plant species observed. Other results indicate that an at least occasional preference for low water content, diseased or damaged plant tissue is widespread in the Acridoidea (Lewis, unpubl.; Smith, 1939; Anderson & Wright, 1952; Gangwere, 1961; Kaufmann, 1968; Mulkern et al., 1969; Ueckert & Hansen, 1971; Bernays et al., 1977; Boutton, 1980). Determining the proximate basis of these preferences can increase our understanding of the nature of plant defence to acridids, and of acridid nutritional and sensory physiology.

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A study of the preference by *M. differentialis* for sunflower leaves from wild and cultivated plants wilted either by girdling (Lewis, 1979), withholding water from potted plants or by excision and air drying (Lewis, 1981) indicates that the preference is a strong one, a majority of grasshoppers feeding only on the wilted leaf. All instars demonstrated the preference, unless food- or water-deprived for 24 h.

Grasshoppers given continuous access from hatching to a mixed diet of excised wilted and turgid leaves show increased survival, growth rate, fecundity and offspring egg weight as compared with ones raised on turgid leaves only. The growth difference appears in the first instar and is due to both increased dry matter consumption and increased efficiency of conversion of ingested food by mixed diet grasshoppers (Lewis, 1981, in prep.). This growth effect and preference may be related, but are not necessarily brought about by the same altered leaf characteristic. The grasshoppers may be responding to a stimulus that signals that wilting has occurred, but which is not itself necessarily beneficial.

This paper focuses on the preference. The effects of leaf water content, plant vigour and leaf rehydration on the preference are described, and the information they provide about the possible cue or cues is considered.

Materials and methods

Grasshoppers Grasshoppers for testing were hatched in the laboratory from egg pods laid by females caught at the study sites in central Texas where the original observations were made. Stock cages were populated with the offspring of 6–12 females and test grasshoppers were taken from a minimum of two stock cages for each test. The cages were placed in a growth room with mixed incandescent and fluorescent ligth at a 14-hour photoperiod, 50% RH and temperatures of 33°C day and 23°C night. Each cage contained a mixture of wilted and turgid sunflower, a petri dish of bran, and water for drinking. Third through fifth instars of either sex were used in testing as previous experiments showed that the preference did not differ significantly among sexes or instars. Grasshoppers were not deprived of food or water before the test unless specified below.

Plants Wild plants were grown from seed collected the previous year at the study sites and sown into disturbed soil at the field station. Leaves from volunteer plants were also used. Seed of the cultivar HY 896 was sown in the cultivated garden at the field station. Fertilizer (Fertilome® 11-15-11) was applied at the rate of 0.2 kg/8 m row. Plants were thinned and watered as needed.

Preference tests In standard preference tests, individually caged grasshoppers were offered a two-way choice between a turgid excised leaf and one treated as required by the experiment. Tests were conducted in the growth room at 33°C for 2 h. Test cages consisted of gauze-topped clear plastic cylinders 8 cm in diameter and 7 cm in height. The cages were placed on 10 cm square plastic platforms with a small hole in the center. The platforms were placed over vials of water. Sections of stem containing leaves to be tested were cut in the field and immediately immersed in water. The petioles of leaves to be tested as turgid were cut under water and

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placed through the holes in the platforms into the vials of water. Turgid leaves treated in this way maintained full water content for at least two days, 800 g/kg for leaves from wild plants and 860 g/kg for leaves from cultivated plants. The total elapsed time from collection of leaves in the field to termination of the preference test seldom exceeded 3 h. Ryan (1979) showed that similarly treated leaves did not accumulate proteinase inhibitor inducing factor, a wound response, for several hours.

The petioles of leaves to be tested as wilted were severed and the leaves wilted in air to the water content required by the test. These leaves provide a good model of the girdled leaves preferred in the field. In both cases, breakdown products of proteins and carbohydrates, and plant hormones cannot be exported down the stem, and so increase in concentration in the leaf.

Amounts of the two types of leaves eaten were usually determined visually, as there were large differences in most cases. When the difference was not clear, the dry weights ingested were then determined. The number of grasshoppers consuming more wilted tissue than turgid was compared with the number consuming more turgid than wilted, using the non-parametric sign test (Dixon & Massey, 1969).

Water content of preferred leaves To determine at what water content wilted leaves first become preferable to turgid ones, grasshoppers were first food- and water-deprived for 1 h. This is the typical interfeed length, and insures that the grasshoppers are sufficiently motivated to begin searching for food before the leaves begin to wilt, but are not so hungry as to lose discrimination. A pair of turgid wild sunflower leaves was placed in the preference cage. Immediately before the grasshopper was placed in the cage, the petiole of one leaf was severed. As soon as the grasshopper had clearly made a choice and begun to feed, the leaves were weighed. They were then dried and re-weighed to determine the water content at the time of choice.

To establish the lower limit of the water content that would elicit the preference, grasshoppers were offered a choice between a wild sunflower leaf dried at 35°C, 50% RH to constant weight and a turgid leaf in a standard preference test for 2 h. To determine if the grasshoppers would feed on a turgid leaf as a water source, the same choice was offered over 24 h to two groups of grasshoppers, the members of one group receiving water in a small dish and the others receiving no water.

Plant vigour Wild field plants that were less vigourous in general appearance than nearby ones were selected for testing. These plants were significantly different (P < 0.001, t-test) from more vigourous plants in several parameters. Vigourous plants were taller, had thicker stems and more branches and inflorescences for their height. As vigour may be associated with resistance to *M. differentialis* (Harvey & Hackerott, 1976), grasshoppers were offered wilted and turgid leaves from these less vigourous plants in the standard choice test. Since nitrogen fertilization and thinning bring about vigour in cultivated plants, leaves from 'HY 896' plants grown without fertilizer and thinning were tested in the standard manner.

The results were compared with those from leaves taken from fertilized, thinned plants of the same age in an adjacent row. The total nitrogen content of each of

three 1-gram samples of dried leaves from fertilized and not fertilized, and vigourous and less vigourous plants was determined by the Kjeldahl method.

Rehydration Leaves from cultivated plants were collected in the standard manner. Both leaves from a pair were weighed, and one wilted in air to a water content of about 680 g/kg. Both leaves were then immersed in water to rehydrate the wilted leaf and to expose the turgid leaf to water for the same amount of time. The leaves were then weighed again after the wilted leaf had rehydrated, usually in about 20 min. In all cases, the rehydrated leaf regained 100% of its lost weight. The puncture resistance of 10 leaves of the two types was determined with a leaf penetrometer (Cherrett, 1968).

Results

The water contents of wilted leaves that will elicit the preference are bracketed by the results in Tables 1 and 2. Grasshoppers first distinguish wilted from turgid leaves when their water contents differ by an average of only 50 g/kg. The preference is also exhibited when the choice is between turgid leaves and ones with minimal water content (Table 2). Grasshoppers offered this latter choice over 24 h, rather than the 2 h of the test, do eat a certain amount from the turgid leaf (Table 3) if no free water is available, but eat less of it in the presence of water.

The preference for wilted leaves is not shown when the test leaves are taken from wild plants characterized as less vigourous or from unfertilized cultivated plants (Table 4). The total nitrogen content of vigourous and less vigourous leaves did not differ significantly, while that of leaves from fertilized plants was higher than that from unfertilized plants i.e., 49.1 ± 1.5 and 42.2 ± 0.6 g/kg respectively.

Grasshoppers prefer leaves that have been wilted and rehydrated to matched turgid leaves (Table 5). Leaf hardness did not differ significantly between the two types of leaves.

Table 1. Minimum wilting of sunflower leaves required to elicit preference of *Melanoplus differentialis* nymphs.

n	Mean water content ± S.E. (g/kg) at time of choice		P, Wilcoxon paired
	wilted leaf	turgid leaf	
17 wilted leaf chosen	758 ± 15	810 ± 6	0.001
6 turgid leaf chosen	782 ± 21	782 ± 13	ns

Table 2. Proportion of *Melanaplus differentialis* nymphs preferring turgid (T) or dehydrated (D) wild sunflower leaves.

n	D only	D > T	D = T	T > D	T only	P, sign test
18	0.39	0.28	0.17	0.17	0	0.05 D > T

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Table 3. Relative consumption (%) of turgid (T) and dehydrated (D) wild sunflower leaves by *Melanoplus differentialis* nymphs with continuous access for 24 h.

Treatment	n	$\bar{x}$ dry matter $\pm$ S.E.	
		T	D
No free water	19	0.27 $\pm$ 0.02	0.73 $\pm$ 0.02
Free water	10	0.09 $\pm$ 0.03	0.91 $\pm$ 0.03
			$P\_$ 0.001, t-test

Table 4. Proportion of *Melanoplus differentialis* nymphs preferring wilted (W) or turgid (T) leaves.

	n	W only	W $>$ T	W = T	T $>$ W	T only	P, sign test	
Vigorous wild plants	20	0.70	0.15	0	0.05	0.10	0.01	W $>$ T
Less vigorous plants	18	0.28	0.22	0.05	0.11	0.33	ns	
Fertilized 'HY 896'	13	0.69	0.23	0	0.08	0	0.01	W $>$ T
Unfertilized 'HY 896'	30	0.33	0.30	0	0.23	0.13	ns	

Table 5. Proportion of *Melanoplus differentialis* nymphs preferring rehydrated (R) or control (C) cultivated sunflower leaves.

n	R only	R $>$ C	R = C	C $>$ R	C only	P, sign test
15	0.67	0.13	0	0.13	0.07	0.05 R $>$ C

Discussion

These results, in addition to further specifying the conditions of the preference for wilted sunflower leaves, can be used to make preliminary inferences about the leaf property responsible for it. *M. differentialis'* preference for wilted leaves of a variety of plant species does not necessarily have the same basis in all cases. Within sunflower, leaves wilted by various agents and to different water contents may also be preferred for different reasons.

Wilted leaves may have lower levels of chemical deterrents, higher levels of phagostimulants, or both. It is less likely that wilted leaves are chosen for purely physical reasons. Increases in dry matter per unit volume have been associated with increased hardness (Cherrett, 1968). Wild sunflower leaves have increased hardness, as measured by puncture resistance, as water content decreases, until dehydration,

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at which point the leaves become brittle and easily punctured (Lewis, 1981). The preferred rehydrated leaves (Table 5) did not differ in hardness from turgid controls, indicating that the preference in this case is also not due to decreased hardness. These results are suggestive only, as the extent to which puncture resistance reflects hardness to grasshoppers is unknown.

If the stimulus is wholly or in part chemical, previous work on acridid host choice indicates that it may be either a nutritive or non-nutritive chemical or chemicals, above, on and/or within the leaf (Slifer, 1955; Chapman, 1977; Bernays & Chapman, 1978). Preliminary observations suggest that the turgid leaves are rejected only after nibbling, but before a significant amount of leaf material is ingested.

Water is the chemical most obviously different in concentration between the two leaves. *M. differentialis* may be susceptible to water-loading, as has been suggested for other acridids (Hill et al., 1968; Boys, 1978; White, 1978; McKinlay, 1981). McKinlay (1981) suggests that the large increases in dry matter per unit volume associated with small decreases in water content may be responsible for the improved performance of *M. sanguinipes* raised on mixed diets of fresh and dried food. Water may also interfere with efficient digestion in some way. Nymphs from stock cages fed a turgid leaf ate meals of equal water content but significantly lower dry matter than ones fed a wilted leaf (water content 740 g/kg; Lewis, 1981). This may result from a behavioural regulation of water intake, or from a deterrence of turgid leaves due to another cause. Water and food of high water content are deterrent to non-deprived acridids (Sinoir, 1968; Kendall & Seddon, 1975; Barton-Browne & van Gerwen, 1976; Bernays, 1977). Whether or not *M. differentialis* can detect the small differences in water or water vapour between the leaves tested in Table 1 is unknown.

In any case, some of the results reported here suggest that turgid leaves are not rejected simply because of their high water content:
— Grasshoppers apparently adopted the rather unusual behaviour of drinking water (Bernays, 1977) rather than obtaining it from turgid leaves (Table 3).
— Acceptability of turgid leaves from less vigourous and unfertilized plants (Table 4) also suggests that high water content alone is not sufficient for deterrency. These turgid leaves did not differ significantly in water content from rejected turgid leaves of vigourous and fertilized plants.
— Grasshoppers preferred rehydrated leaves to turgid ones (Table 5). Assuming that the cells on or near the leaf surface of rehydrated leaves have not been differentially altered so as to have lower water contents, these results are a further indication that water alone is not the cue.

The deterrents to *M. differentialis* in sunflower and other plants have not been identified. There is also insufficient information available on stress effects on levels of possible deterrents. Certain kinds of drought stress and low vigour increase chlorogenic acid (Del Moral, 1972; Koeppe et al., 1976), a phenolic compound whose effect on preference and growth of acridids is unknown. Low vigour or wilting may decrease the concentration of some deterrents, as is the case in cassava: nymphs of the grasshopper *Zonocerus variegatus* will only eat wilted leaves, presumably because of reduced production of hydrogen cyanide (Bernays et al., 1977).

Phagostimulants for *M. differentialis* have also not been completely specified.

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Candidate substances include both nutrient and non-nutrient chemicals. Wilting does increase the amounts of certain amino acids (Jones et al., 1980) and under certain conditions, sugars (Levitt, 1972), but it is unlikely that the concentrations increase rapidly enough in leaves stressed for short periods of time to stimulate feeding (Stewart & Larher, 1980). The effect on feeding of wilt-induced increases of other chemicals, such as ethylene or abscisic acid, is unknown at this time.

Whatever the stimulus for the preference proves to be, these results have certain ecological implications. They suggest that low vigour and certain stresses in plants may influence the feeding behaviour of generalist acridids. These insects may be choosing leaves which do not appear to differ from healthy, turgid leaves, but which in fact are either only slightly wilted or have undergone cycles of wilting and rehydration. These may be examples of the sort of determinants of insect intraspecific host choice which are frequently invisible to the investigator in the field.

Acknowledgments

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Plant growth hormones affect grasshopper growth and reproduction

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Abstract

Three plant growth hormones (PGHs), gibberellic acid (GA_3), auxin (indole-3-acetic acid, IAA) and kinetin, usually associated with promotion of growth in young plants, were added, each at two concentrations, to distilled water with which leaves of perennial western wheatgrass *Agropyron smithii*, were watered as they were offered as food plants to paired adult grasshoppers *Aulocara elliotti*. GA_3 at 18 mg/l, IAA at 20 mg/l and kinetin at 20 mg/l distilled water significantly raised the fecundity and increased the number of viable eggs compared to those laid by the control females fed untreated wheatgrass. At all of the concentrations tested these plant hormones also significantly increased the longevity of females compared to the mean of the control females. These results, together with the earlier finding that abscisic acid (ABA), a growth-inhibiting plant hormone, retarded growth and decreased reproduction of *A. elliotti* when added to wheatgrass at 6 mg/l distilled water, support the hypothesis that the seasonal correlation of growth and the success of reproduction of this insect is significantly influenced by changing concentrations of these PGHs in its host plant, perhaps by their direct effect on DNA synthesis in insect cells.

Key-words: gibberellic acid, auxin, kinetin, reproduction, longevity, *Aulocara elliotti, Agropyron smithii,* fecundity, plant growth hormones

Recent research has shown that certain plant substances at specific concentrations are important in establishing seasonal patterns of growth and reproduction in herbivores that ingest them. In microtine rodents, for example, the cyclic carbamate 6-methoxybenzoxazolinone, derived from winter wheat seedlings, triggered the seasonal onset of estrus (Berger et al., 1981) whereas phenolic compounds, such as cinnamic acid and ferulic acid in mature host plants, caused cessation of reproduction in these mammals (Berger et al., 1977). In a previous paper (Visscher, 1980) I suggested that phytophagous insects may use the changing concentrations of the plant growth hormones (PGHs) in their host plants to establish phenological correlation with their environments. Experiments with the rangeland grasshopper *Aulocara elliotti* (Thomas) presented and discussed here, as well as studies with other insect species in my laboratory, suggest that not only the periodicity, but also the extent of growth and reproduction in insects may be significantly affected by the concentrations of PGHs in their host plants.

Changes in the fecundity and egg viability of six geographically isolated wild populations of *A. elliotti* in Montana have been studied for more than a decade in my laboratory. This grasshopper feeds largely on a drought-resistant perennial, western wheatgrass *Agropyron smithii* Rydb. It lays eggs during the summer which overwinter and hatch in spring, thus having but one generation each year. Meteorological data collected near the population sites, when compared with the annual changes in reproductive performance, revealed a high correlation between above-average winter precipitation and subsequent production of larger numbers of viable eggs. Years of low fecundity and low egg viability followed periods of fall and winter drought (Visscher & Wrobetz, unpubl.). This suggested that the fecundity of *A. elliotti* somehow was affected by the environmental stresses on its host plant. Western wheatgrass was then grown in three different environments and fed to adult *A. elliotti* reared at warm or cool temperatures. Females fed grass grown at cool temperatures laid viable eggs at a rate faster than females fed on grass grown in the field or at warm temperatures (Visscher et al., 1979). Because drought-stress, high temperatures and senescence can induce the production of the growth-inhibiting hormone abscisic acid (ABA) in plants (Milborrow, 1978), it seemed possible that high titers of this plant hormone might act as a biochemical signal indicating physiological stress in the host plant to the insect that ingested it, perhaps slowing their growth or inhibiting their reproduction. Indeed, de Wilde et al. (1969) reported cessation of reproduction in the Colorado beetle when adult females were fed on senescent plants.

To test the effects of ABA on the reproduction of *A. elliotti*, leaves of western wheatgrass were watered with solutions of ABA at 6 or 60 mg/l (first dissolved in 10 ml of 95% ethanol, then diluted to 1:1 with distilled water) as they were offered as food plants to adult grasshoppers throughout their reproductive lifespans. Females fed such treated leaves laid an average of only 1.5 viable eggs, as compared with 14.3 eggs per control female. Other females fed *A. smithii* watered with gibberellic acid (GA_3) at a concentration of 60 mg/l distilled water (also dissolved first in 10 ml of 95% ethanol) laid an average of only 1.3 viable eggs over their entire adult lifespans (Visscher, 1980). This result was unexpected since Ellis et al. (1965) reported that GA_3 added to the diet of senescent vegetation fed to female desert locusts *Schistocerca gregaria*, restored their sexual maturation. Salama & El-Sharaby (1972), however, also found that GA_3 acted as a sterilant, so it was speculated that perhaps reproductive performance in insects is related to the concentrations of the plant growth hormones (PGHs) present in the host plant. If this hypothesis was correct, then at certain concentrations all of the growth-promoting plant hormones should stimulate the growth and reproduction of insects because they are present in young growing vegetation, just as ABA could inhibit reproduction at certain concentrations.

Materials and methods

To examine this hypothesis, 23-33 pairs of newly-moulted adult *A. elliotti* were fed western wheatgrass collected from a field site near Bozeman three times each week and placed into a solution of one of three PGHs. Approximately 10 ml of each

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treatment solution containing either GA$_3$ at 6 or 18 mg/l, auxin (IAA) or kinetin at 10 or 20 mg/l distilled water was added to grass in the food vials as they were placed in the cages. Grass in the control regimen was watered with 10 ml of a solution containing 1 ml/l of 95% ethanol (the solvent for GA$_3$ and IAA). Kinetin was dissolved in 0.5 ml HCl concentration 3 mol/l before dilution with distilled water at both concentrations.

Soil in the cage bottoms (metal pans of diameter 9 inch) was sifted daily to collect the egg pods, and these were incubated for 30 days at 25°C according to published methods (Visscher, 1971). The eggs were then removed from the pods, fixed in Bouin's solution, rinsed, stored in 70% ethanol and subsequently examined microscopically to determine whether they contained viable embryos. The mean numbers of eggs and viable eggs laid and the longevities of females in each of the regimens were calculated and compared by analysis of variance (ANOV). The mean square error (s^2) was based on ANOV. Comparisons of treatment groups to the control were based on the calculation $t = (\bar{x}_c - \bar{x}_i)/[s^2 (1/n_c + 1/n_i)]^{1/2}$ where $\bar{x}$ is a group mean, s^2 the mean from ANOV, n the group size and the subscripts c and i are the control and treatment groups (Student's t-test).

Results and discussion

The numbers of fertile females in each regimen and the data concerning their reproduction and longevities are presented in Table 1. Females fed wheatgrass watered with GA$_3$ at 18 mg/l and IAA at 20 mg/l distilled water laid significantly more viable eggs than did the control females ($P < 0.05$ and < 0.01 respectively). Females fed grass watered with kinetin at the 20 mg/l concentration likewise produced more than twice as many viable eggs as did the control females, but this difference was less significant ($P < 0.08$) because of the wide variability among females in that regimen. In all regimens in which females were fed PGHs at the

Table 1. Plant growth hormone effects on the reproduction, egg viability and longevity of the grasshopper *Aulocara elliotti* (O.W. Population, 1980).

Treatment group	Total females	Females laying eggs	Mean eggs/female	Mean viable eggs/female	Mean adult female longevity (days)
Control	33	22	17.00	9.18	45.68
GA$_3$–6 mg/l	23	14	29.21	17.71	52.71
GA$_3$–18 mg/l	23	16	28.06	19.44*	57.63*
IAA–10 mg/l	23	19	21.00	14.37	55.47
IAA–20 mg/l	23	14	36.14**	22.93**	59.00*
Kinetin–10 mg/l	23	16	25.25	15.37	52.19
Kinetin–20 mg/l	23	11	28.28	19.18	61.00*
Mean square error	–	–	402.80	242.60	297.20

* $P < 0.05$
** $P < 0.01$

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lower concentrations, both the mean number of eggs and the mean number of viable eggs laid by females exceeded those laid by control females. The mean longevities of females fed grass watered with PGH solutions at the higher concentrations were significantly longer than those of control females ($P < 0.05$).

Stimulation of reproduction by GA_3 was reported by Scheurer (1976) in aphids fed on treated *Vicia* isolates. By contrast, GA_3 added at 60 mg/l in water to leaves of wheatgrass fed to *A. elliotti* significantly decreased the number of viable eggs from 14.3 to 1.3 per female (Visscher, 1980), whereas in the present experiment GA_3 at lower concentrations, 6 and 18 mg/l, added to that grass increased the production of viable eggs in that grasshopper. Such paradoxical effects on insect reproduction have also been observed when other PGHs were added to their diets. While IAA significantly increased the reproduction of *A. elliotti* in the present experiment and that of the corn leaf aphid (Oka & Pimentel, 1974), analogues of IAA or related compounds (3-methylindol, 2,4,5-T and indole) decreased the progeny of *Heliothis* sp. fed on artificial diet (Guerra, 1970). Moreover, Guerra found that N-6-benzyladenine, a synthetic cytokinin, fed to *Heliothis* significantly decreased the number of viable eggs laid, whereas kinetin, also a synthetic cytokinin, increased the reproductive performance of *A. elliotti* at both the concentrations fed here. Thus, not only have opposite reproductive responses been observed when a given PGH was fed to different insect species, but also when a single PGH was fed at different concentrations to a single insect species (*A. elliotti*). This suggests that insects respond physiologically in a dose-related manner to dietary PGHs. However, because host plants and the so-called artificial diets, the latter often containing seed meals or other plant materials, may contain endogenous PGHs in varying and unknown amounts, it is not yet known which concentration of any PGH is effective either in stimulating or inhibiting reproduction. Thus although one could perhaps add a known amount of a PGH to a cellulose disk in order to quantitate the amount consumed, until a defined diet completely devoid of the PGHs other than those to be tested can be used, endogenous PGHs complicate the task of identifying the effective concentrations. Finally, bacteria and fungae inhabiting the insect gut or haemocoel may contribute PGHs to the insect, adding to experimental amounts or perhaps introducing opposite effects of those anticipated from the experimental substance.

When PGHs are fed to insects by adding them in water to the host grass, as they must be for *A. elliotti* which thus far cannot be reared on a defined medium, the possibility exists that the insect response is elicited by some secondary plant substance induced by the PGH. When significant reproductive changes were obtained by feeding 6-benzyladenine to *Heliothis* in an artificial diet (Guerra, 1970), the effect seems unlikely to have come from an induced plant substance. There is evidence, as suggested by Osborne et al. (1968), that the PGHs may act through their effects on the insect neuroendocrine system or perhaps directly on insect cells.

De Man et al. (1981) injected ABA into the fleshfly *Sarcophaga bullata* and found that vitellogenin synthesis was inhibited. When sugar impregnated with 5 mg/g ecdysterone was fed thereafter, the inhibition was overruled. Recent experiments in my laboratory have demonstrated direct effects of the PGHs on the rate of DNA synthesis in insect cells in vitro. In cell suspensions derived from embryonic *A.*

elliotti or *Melanoplus sanguinipes* Fabr. and cultured with tritiated thymidine in Mark's M-20 medium (GIBCO Laboratories, Grand Island, N.Y. 14072), GA_3 and kinetin added at concentrations of 10^{-2} to 10^{-8} µg/ml to the medium significantly increased the incorporation of thymidine into DNA at concentrations of 10^{-2} to 10^{-6} µg/ml (Visscher, unpubl.). These results and those of De Man et al. suggest that dietary PGHs may regulate insect growth and reproduction directly by altering the rate of DNA synthesis and/or the rate of synthesis of the insect moulting hormone.

Experiments conducted in collaboration with Jurenka could support either possibility. The growth rate and survival of nymphal *A. elliotti* fed on wheatgrass harvested in July or August was compared with that of nymphs fed grass harvested in May. In two regimens ABA was added at 6 or 60 mg/l in distilled water to grass harvested in May. The growth and survival of nymphs fed treated May grass were significantly slower and lower than that of nymphs fed untreated July grass, but similar to that of nymphs fed untreated drought-stressed August grass (Visscher & Jurenka, unpubl.). Analysis of the ABA content of treated May grass showed the concentrations of ABA to be similar to those found in untreated August grass (Lewis et al., unpubl.). The close correlation between the concentrations of ABA in treated May grass with that found in drought-stressed August grass, together with the similarly inhibited patterns of growth in nymphs fed these grasses, provides at least preliminary evidence that ABA may be the naturally occurring insect growth inhibitor present in the stressed August grass.

Another naturally occurring plant growth inhibitor, ethylene, either retarded or accelerated nymphal growth in *Melanoplus sanguinipes* depending on the period of exposure or the dose received (Chrominski et al., 1982). Edwards (1966) found that ethylene at high concentrations (40 and 80 %) significantly retarded the growth of house crickets.

It seems significant that compounds belonging to all five classes of PGHs have been shown to alter insect growth and/or reproduction. In this paper, significant responses were found to be concentration-related. Because the interrelationships between the various PGHs and the secondary plant substances are uncertain, it is not known how interactions of the former with the latter might affect insects. Further, because the PGHs interact with each other in plants to produce effects different from those each may cause independently, it seems possible that the PGHs might also interact in insects. It can only be speculated whether the PGHs will prove equally important to the growth, reproduction and ageing processes of other phytophagous animals.

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Plant-mediated variation in herbivore suitability and parasitoid fitness[1]

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Abstract

Most available studies on the role of variation in host herbivore suitability on parasitoids provide indirect evidence that individuals of a given species vary depending on the quality of their diet and that these differences have corresponding effects on their parasitoids. A few studies have provided direct evidence that dietary deficiencies or allelochemicals in the food of herbivores have a major impact on their parasitoids. Our preliminary data indicate that in the tobacco hornworm survival of its parasitoid, *Apanteles congregatus*, is affected by the nicotine in the hornworm's diet and that the nicotine is retained in the parasitoid. Some of the variables which may influence this type of tri-trophic level interaction are discussed. The implications to biological control of the variation in host suitability and its effects on parasitoids are noted.

Key-words: parasites, secondary plant metabolites, nutrition, herbivore suitability, *Apanteles congregatus*, nicotine, tobacco, phenols, *Myzus persicae*, alkaloids

Host suitability is a major component of the approximately five phases of host selection as outlined by Salt (1935), Flanders (1953), Doutt (1959), Vinson (1975) and others. It can be defined as the degree to which the environment within the host provides the requisites for successful development and emergence of fertile adult parasitoids. Variation in herbivore host quality (or suitability) results in great part from quantitative and qualitative differences in the nutritional and allelochemical composition of the herbivore.

The classic studies of Salt (1941) demonstrated that interspecific variation in hosts can have some dramatic and potentially significant effects, presumably due to an insufficiency of food for developing parasitoids. Alternatively, an important source of variation in host suitability may result from intraspecific differences. Intraspecific variation in herbivore hosts, particularly polyphagous herbivores, may have a significant impact on associated parasitoids.

A variety of factors may induce significant variation in the quality of host individuals of a given species. For example, gypsy moth larvae, from eggs collected from three types of populations ranging from innocuous (or endemic) to outbreak, reared on an artificial diet under identical conditions become in quantitatively and qualitatively different individuals. Significant differences in developmental parameters including differences in female and male pupal weight as well as fecundity result depending on whether eggs were originally deposited by females from endemic, release or outbreak populations. Differences in hemolymph amino acids and carbohydrates also can be demonstrated in individuals from each population type. These differences have an important influence on parasitoids, since the parasitoid progeny of *Brachymeria intermedia* and *Coccygomimus turionellae* differ in weight, size, development time, etc. depending on the type of host in which they develop (Greenblatt & Barbosa, 1981).

The various factors associated with different population densities are not the only factors capable of inducing host herbivore variation. Indeed, one of the most important factors responsible for variation in host herbivore suitability may be the plant it consumes. The objective of this paper is to focus attention on the importance of the nature of the diet of herbivores on parasitoids, namely, host plant-mediated alterations in host herbivore quality.

The scarcity of data on this tri-trophic level interaction make it difficult to make any rational unifying generalizations. Nevertheless, in order to evaluate the current status of our understanding of these interactions, the key issues that must be addressed and the potential impact of these interactions, it is important to selectively review the types of information that are available and ponder the variables that may influence this tri-trophic interaction. With this perspective, suggestions can be made on how sorting out the relative role of variation in host suitability, particularly related to the influence of allelochemicals can be of importance to biological control.

Parasitoid survival and development

In most available studies no cause and effect relationships are established between changes in host quality and variation in parasitoid survival and development, although numerous suggestions have been made. Cheng (1970) suggested that rapid parasitoid development in certain hosts fed on specific plants provided protection against encapsulation because of the differential effectiveness of such a host defense on parasitoid larvae of different sizes. Mortality due to encapsulation of the larvae of the parasitoid *Lypha dubia* within its host *Operophtera brumata* was higher in oak-fed winter moths compared to those in larvae fed on hawthorn, blackthorn and hazel. Flanders (1942), Lange & Bronskill (1964) and Pimentel (1966) have suggested that differential parasitoid development may be due to an inappropriate balance or absence of key nutritional compounds within the host which, in turn, reflect differences in herbivore diets. *Habrolepsis rouxi*, an encyrtid parasitoid of *Anonidiella aurantii* (the California red scale) rarely completes development when its host is reared on sago palm compared to when it is reared on citrus (Flanders, 1942). *Musca domestica* (the house fly) reared on chemically defined diets differs in its ability to inhibit the development of the parasitoid *Aphaereta pallipes*. In addi-

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tion, inhibition of parasitoid embryogenesis in hosts of one strain was less frequent when hosts were reared on the diet upon which their colony had been previously maintained (Lange & Bronskill, 1964). Similarly, when *Nasonia vitripennis* are reared on house flies reared on various media, differences were observed in average female life span and percent parasitism (Pimentel, 1966). Greenblatt & Barbosa (1981) suggested that changes in parasitoids may be due to host plant-mediated differences in the levels of amino acids and carbohydrates in gypsy moths *Lymantria dispar*. Differences in larval development, pupal weight, biochemical composition in herbivore hosts reared on different plant species resulted in differences in the metabolism, emergence weight, size, sex ratio and development time of the parasitoids, *Brachymeria intermedia* and *Coccygomimus turionellae* (Greenblatt & Barbosa, 1981).

Finally, others have speculated or their work has suggested that the quality and quantity of allelochemicals may play a major role in variation in host herbivore quality (Morgan, 1910; Gilmore, 1938a,b; Smith, 1978; Pasteels, 1978; Blumberg & DeBach, 1979). Sub-optimal development of *Anonidiella aurantii* on sago palm compared to yucca, lemon and castor bean had detrimental effect on the development, survival, sex ratio and size of its parasitoids, *Habrolepsis rouxi* and *Comperiella bifasciata* (Smith, 1957). Host plants which were optimal for *Spodoptera littoralis* (the cotton leaf worm) were least suitable for its parasitoid, *Microplitis rufiventris*. Gossypol was suggested as having a role in the interaction (Altahtawy et al., 1976). Development of the parasitoid *Bracon gelechiae* proceeds normally in gram (chick pea) *Cicer arietinum* fed larvae. However, when tobacco fed larvae were used as hosts parasitoid larvae died within 3 days after feeding within hosts (Narayanan & Rao, 1955). Regardless of the mechanism causing variation in host herbivore suitability these studies have demonstrated that such variability can lead to changes in parasitoid survival and development, size, sex ratio, percent parasitism, fecundity, percent emergence and morphology.

Only a few studies have directly demonstrated the effects of nutritional compounds of host diet on parasitoids or the role of allelochemicals. Development of the immature stages of the parasitoid *Aphelinus asychis* was delayed and emerging adults were small and died sooner on hosts on deficient diets. Diets varied in sucrose content from 150 (i.e., a complete diet) to 37.5 g/kg and in iron content from 0.023 g/kg to 0 g/kg (Zohdy, 1976). Campbell & Duffey (1979) demonstrated that tomatine (an allelochemical in tomato) incorporated in the diet of *Heliothis zea* resulted in prolonged larval development, reduced pupal eclosion, smaller size and reduced adult longevity of the parasitoid *Hyposoter exiquae*. Our data suggest that in the tobacco hornworm survival of its parasitoid *Apanteles congregatus* is affected by the nicotine in the hornworm's diet. The parasitoids take in and retain nicotine in their bodies (Table 1). Nicotine also appears to be incorporated in the cocoons. The number of parasitoid larvae that successfully emerge from their hornworm hosts to form cocoons differ in diets with nicotine compared to those without (Table 2). However, the development time for those that do emerge is not significantly different from control parasitoids. These results confirm and extend the results of Thurston & Fox (1972). Other studies, in part based on indirect evidence, have suggested that parasitoids and some predators may be able to sequester allelochemicals

Table 1. Uptake of radio-active labeled nicotine by *Apanteles congregatus*, a gregarious para-sitoid of the tobacco hornworm[1,2].

Tissue	Mean cpm/mg of sample	Mean cpm/mean wt of individual
Adult	5.7423 ± 0.7494[3]	190.48 ± 30.4211
Cocoons	2.8862 ± 0.369	106.17 ± 28.9660

1. Unpublished data (Barbosa et al.).
2. All counts above background.
3. Standard error.

like cardenolides, cyanogenic and cardiac glycosides, pyrrolizidine alkaloids, etc. (Jones et al., 1962; Rothschild et al., 1973; Rothschild et al., 1977; Benn et al., 1979).

Host herbivore variation

Generalizations on the role of host herbivore variation will be difficult to estab-lish until the types of interactions that occur and the factors that influence this phe-nomenon are understood. The variables influencing these interactions are numerous but for the purpose of illustration let us consider three major ones which focus on allelochemicals. These three variables are the ways in which herbivores deal with allelochemicals, the feeding sites of larval parasitoids and the host range of para-sitoids i.e., whether a parasitoid is a generalist or a specialist. All these factors as well as others may determine the degree and nature of exposure of developing para-sitoids to allelochemicals and their potential capacity to deal with allelochemical-mediated variation in hosts.

Allelochemicals There are a variety of methods by which herbivores deal with tox-ins. One of these is sequestration (Duffey, 1980). Although many species have been found to sequester allelochemicals throughout their bodies (Morris & Thompson, 1963, 1964; Aplin & Rothschild, 1972; Isman et al., 1977a; Daloze & Pasteels, 1979; Duffey, 1980) others like certain species of grasshoppers, Lepidoptera or sawflies in the subfamily Perginae store allelochemicals in special glands or in foregut diver-ticula (Hollande, 1923; von Euw et al., 1967; Morrow et al., 1976). Still others like

Table 2. Influence of nicotine on *Apanteles congregatus*, a gregarious parasitoid of the to-bacco hornworm.

Treatment (hornworm diet)	Number of cocoons formed	Number of parasitoids emerging	Development time in host (days)	Emergence from cocoons (%)
Control	283.0 ± 56.6[1]	241.6 ± 47.7	27.0 ± 1.2	85.5 ± 1.9
0.1% nicotine	84.7 ± 16.7	73.0 ± 14.2	24.5 ± 1.7	86.5 ± 2.5

1. Standard error.
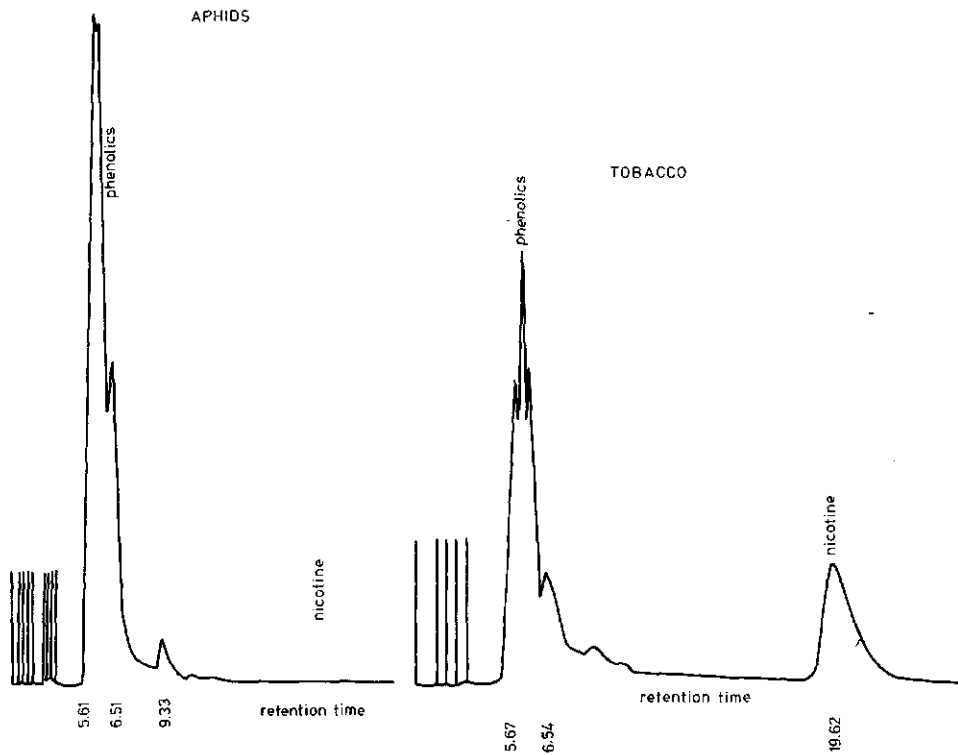
Fig. 1. Chromatogram of allelochemicals in *Myzus persicae* aphids and the tobacco leaves on which they have fed.

Oncopeltus fasciatus sequester cardenolides from host plant seeds in a series of bilateral dorsal thoracic and abdominal spaces (Isman et al. 1977b; Duffey et al., 1978). The critical point is that the details of the location of allelochemicals may determine whether or when parasitoids are exposed to these allelochemicals.

A second mechanism of dealing with allelochemicals is avoidance. The green peach aphid *Myzus persicae*, has been reported to avoid the toxic effects of the nicotine in its tobacco hosts by restricting its feeding to phloem which contains no nicotine (Guthrie et al., 1962). And indeed, the nicotine found in the plant is not found in the aphid (Figure 1). However, our preliminary evidence suggests that a phenolic compound or compounds do occur both in the plant and the aphid (Figure 1). Rather than avoidance we may have an example of a trade-off among allelochemicals. Thus, although its parasitoids may not have to deal with one allelochemical, nicotine, because the host behaviorally avoids it, they may nevertheless have to deal with one or more other allelochemicals, in this example, phenolics.

Herbivores may deal with allelochemicals by egestion or metabolic degradation. The tobacco hornworm *Manduca sexta*, the cabbage looper *Trichoplusia ni* and the tobacco budworm *Heliothis virescens*, are examples of species which utilize fast and

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efficient egestion systems (Self et al., 1964) while species like the tobacco wireworm *Conoderus vespertinus*, cigarette beetle *Lasioderma serricorne* and differential grasshopper *Melanoplus differentialis*, have evolved the ability to metabolize nicotine to cotinine and 2-3 additional metabolites (Self et al., 1964). What the parasitoid is exposed to, how much it is exposed to, and for how long it is exposed may all be affected by the method utilized by host herbivores to deal with allelochemicals.

Feeding sites   An important variable is the feeding site of the parasitoid. Although many parasitoids begin feeding in the hemolymph and later proceed to feeding on vital organs many species exhibit feeding in specialized sites. Various tachinid parasitoids feed on brain tissues, supra-oesophageal ganglia or the tissues of the salivary gland. Some proctotrupid species feed in the alimentary tract of host larvae feeding on the fluid contents of its host intestine. Still others enter the midgut and establish themselves within the intersegmented muscle until the host reaches an appropriate stage (Salt, 1968; Shields, 1976). Thus, exposure to allelochemicals depends not only on the fate of the plant allelochemical within the host but also on the location of the parasitoid.

Host range and host suitability   The host range of the parasitoid, i.e., whether it is a generalist or a specialist, may be important in the adaptability of some species to allelochemicals in their hosts. Regardless of the nature of the interactions the existence of this relationship has some important implications to various aspects of the biological control on insect pests. Mass colonization may produce changes in insects which arise through nongenetic processes. These include either conditioned behavior changes or alterations in physiological processes. A major change in host biochemicals or inadequate nutrition can result in altered or probably deficient performance by a colonized insect species (Chambers, 1977). These conditioned changes may become reinforced through reproductive isolation, particularly in long-term colonization. This is of particular importance in parasitoids which have been shown to have the capacity to be conditioned (Arthur, 1971; Vinson, 1975, 1976; Vinson et al., 1977). Thus, the parasitoid that is mass-produced may be ill prepared to deal with the biochemical milieu that it must face within the host in the field and that that milieu may not be the same from host individual to host individual due to the herbivore's diet.

Secondly, the performance or 'effectiveness' of many parasitoid species is often inconsistent or highly variable from place to place and year to year. Clearly, although a process as complex as the dynamics of the interaction between a host and its parasitoid is not explained by one factor the host plant-mediated variation in herbivore suitability may be an important factor in the variable performance of parasitoids or the development of geographic strains. Polyphagous herbivores, indeed, may vary more from area to area than previously assumed if the current hypothesis that highly polyphagous herbivores may be more accurately considered to be localized specialists is correct (Fox & Morrow, 1981).

A final issue is that of the potential interactions between biological control approaches. The use of host plant resistance often relies on increasing levels of al-

lelochemicals responsible for antibiosis. Available data indicate that natural enemies like parasitoids and predators are significantly more sensitive to toxic compounds than their herbivore host (or prey). Thus, as has been suggested by Campbell & Duffey (1979), although the increase concentration in a given allelochemical may increase the mortality of the herbivore it may very well take a greater toll of the parasitoid. It should be clear that the established and potential effects of plants on host suitability and the role of plants in host-habitat and host finding requires that tri-trophic level interactions be an important consideration in breeding for host plant resistance.

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The significance of salicin for a Salix-feeder, Phratora (Phyllodecta) vitellinae

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Abstract

Phratora vitellinae feeds mainly on *Salix* and occasionally on *Populus* spp. Its larvae secrete salicylaldehyde which defends them. It has been suggested that this is derived from salicin found in the leaves of some *Salix*. Our aim was to test this hypothesis, and to assess the possible influence of salicin on host-plant selection. In nature, adults are found mainly on salicin-containing *S. nigricans* and not on *S. cinerea* and *S. caprea*, which do not contain phenolglycosides in the leaves and further have the leaf lower surfaces covered with trichomes. Laboratory choice experiments demonstrated a corresponding preference. After being shaved, however, the leaves of *S. caprea* were readily accepted by the larvae. Larvae developed on shaved *S. caprea* leaves, but did not secrete salicylaldehyde. Addition of salicin to *S. caprea* restored secretion of salicylaldehyde. Salicin is thus a precursor for the larval defensive secretion, but is not obligatory for food acceptance.

Key-words: Phratora vitellinae, salicin, defensive secretions, food preferences, salicylaldehyde, trichomes

Phratora (Phyllodecta) vitellinae (Coleoptera, Chrysomelidae) feeds on *Salix* and *Populus* spp.. Its larvae secrete salicylaldehyde (Wain, 1943). A mechanism for deriving salicylaldehyde from salicin, a phenolglycoside contained in the leaves of some *Salix* and *Populus* spp., was proposed by Wain (1943) and Pavan (1953) but was never verified. One of our aims was to confirm or disprove this hypothesis experimentally. *Salix* spp. fall into at least two groups on the nature of the secondary compounds of their leaves (Hegnauer, 1973). Some species (e.g. *S. nigricans, S. purpurea* and *S. fragilis*) contain phenolglycosides, such as salicin, but no proanthocyanidins in their leaves. The surfaces are usually glabrous. Additionally, there are species (e.g. *S. alba, S. caprea* and *S. cinerea*) with leaves containing no phenolglycosides but rich in proanthocyanidins. The undersurfaces of these leaves are usually covered with trichomes.

In this study, the consequence of the absence of salicin in the food plant on the chemistry of the defensive secretion was examined. Besides, the relative preference of *P. vitellinae* for various native *Salix* spp. has been investigated in the field and in the laboratory. The effect of leaf trichomes and of salicin on food plant choice by

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adults, on larval mortality and its incidence during different instars, and on the relative growth rate of the beetles was especially studied.

Materials and methods

This study was carried out in and around Basel (Upper Rhine valley). For the food plant choice trial, adult females were first fed for 24 h on their favorite food plant, *S. nigricans*. They were then isolated and given the choice between two entire young leaves belonging to two *Salix* spp. They were allowed to feed on them for a further 24 h. At the end of the test, the plant that was more eaten than the other was noted. Each combination of plants was tested 11 to 20 times with different beetles. The results were analysed with a binomial test.

P. vitellinae larvae were reared in 55-millimetre petri dishes lined with moist filter paper, each containing four 14-millimetre leaf discs arranged in a square. The leaf discs were replaced daily, the larvae counted and their developmental stage noted. The dishes were kept at room temperature (20-28°C) with no direct sunlight. Adults were weighed on the day of emergence.

Leaf discs were made from *S. nigricans, S. caprea, S. caprea* with the trichomes removed from the lower surface ('shaved') and *S. caprea* shaved plus salicin. *S. caprea* leaf discs were shaved by brushing carefully the undersurface with adhesive tape. Salicin was applied as a 1% solution to some *S. caprea* discs to approximately 2% of the discs' dry weight. This concentration is of the same order of magnitude as that reported for willow leaves (Hegnauer, 1973).

The secretion of third instar larvae was collected individually in calibrated glass capillary tubes. Special care was taken to empty the glands completely. The height (about 0.05 mm) of the liquid in the capillary was measured under a binocular microscope with a stage micrometer. The full tubes were stored in 0.2 ml hexane in the refrigerator. The exuviae of the third instar were collected after pupation and stored in hexane.

The amount of salicylaldehyde in the secretion was determined by quantitative gas chromatographic (GC) analysis of extracts of the secretion and by comparison with standard curves derived from a reference compound. The amounts given (Table 3) were calculated from two different GC injections. A Hewlett Packard 102 GC equipped with ionization detectors was used, with glass columns of length 1.80 m and of inner diameter 3.5 mm, packed with S.P. 1000. Column temperature was 90°C.

Results

P. vitellinae were counted regularly at the study site. *S. nigricans* supported a large population; e.g., in May, 4 min counts gave an average of 55 ± 30 adults per tree (*n* = 14). Only few beetles were found on nearby *S. purpurea* (5 ± 4, *n* = 3). Virtually no beetles were present on *S. cinerea, S. caprea, S. alba* or *S. fragilis*.

P. vitellinae adults overwinter in groups in sheltered places. In the spring, they colonise young *Salix* trees on which they feed and reproduce. The eggs are laid on the lower leaves of the branches where the larvae feed, pupation occurs in the soil

Table 1. Food plant choice of *Phratora vitellinae* females.

Choice offered A – B	n	A > B	B > A	P (binomial test)
Salix nigricans - S. fragilis	11	7	4	0.274
S. fragilis - S. purpurea	11	9	2	0.033
S. purpurea - S. alba	15	12	3	0.018
S. alba - S. caprea	11	9	2	0.033
S. caprea - S. cinera	11	9	2	0.033
S. purpurea - S. cinera	20	20	0	0.001
S. caprea - S. caprea shaved	10	0	10	0.001
S. caprea shaved - *S. caprea* shaved + salicin	10	5	5	0.623

under the tree and the young emerging summer adults go back up the tree to feed. Host tree selection thus seems to happen annually, in the spring. The May census of the *P. vitellinae* population therefore probably reflects the preferences of the beetles in the spring.

Table 1 summarises the binary choice experiments performed and their results. The actual combinations of *Salix* spp. used were arrived at after preliminary tests and field observations. These results show clear preferences by adult *P. vitellinae* among the young *Salix* leaves of different species. The following rank order of *Salix* spp. is established: *S. nigricans > S. fragilis > S. purpurea > S. caprea > S. cinerea.* Except for the first pair, the differences in palatability between the adjacent species are all significant ($P < 0.05$). The three species having glabrous leaf surfaces and containing salicin are preferred to the three species not containing salicin but rich in condensed tannins and having trichomes on the surfaces of the leaves. Shaved *S. caprea* discs are however readily accepted, and the addition of salicin to shaved *S. caprea* does not influence the choice. Thus the trichomes seem to play an important deterrent role and salicin is not necessary for food plant acceptance by *P. vitellinae*. The factors governing food plant choice will be further examined in a subsequent paper.

A preliminary experiment showed that *P. vitellinae* reared on *Salix* spp. containing no salicin had a higher larval and pupal mortality than those on leaves with salicin. Table 2 summarises subsequent experiments and shows the distribution of mortality with age, and the influence of different food plants. Mortality was high on all plants for still unexplained reasons, but some clear difference appeared among the treatments. The different rates of mortality during the first and the second instars implicate the trichomes of *S. caprea* as a lethal factor. Indeed, the differences were only significant ($P < 0.05$) when comparing the mortality of larvae reared on *S. nigricans* or shaved *S. caprea* to those reared on unshaved *S. caprea* (χ^2-test). The trichomes seem to prevent the small first instar larvae from reaching the leaf surface. The few that reach the second instar are big enough to feed normally. Presence or absence of salicin does not affect mortality during the first two instars. Larvae feed equally voraciously on *S. nigricans* and on *S. caprea* shaved with or without

Table 2. Percentage mortality of *Phratora vitellinae* larvae reared on leaf-discs (number of each instar in brackets). L_1: number of larvae instar 1; L_2: number of larvae instar 2; L_3: number of larvae instar 3; P: number of pupae; Ad: number of adults.

Salix spp.	L_1-Ad	L_1-L_2	L_2-L_3	L_3-P	P-Ad
	L_1	L_1	L_2	L_3	P
S. nigricans	81.1%	45.2%	23.6%	50.4%	8.9%
	(Ad 51)	(L_1 270)	(L_2 148)	(L_3 113)	(P 56)
S. caprea	98.1%	77.7%	25.0%	77.7%	50%
	(Ad 1)	(L_1 54)	(L_2 12)	(L_3 9)	(P 2)
S. caprea shaved	97.3%	50.3%	34.2%	81.6%	55.5%
	(Ad 8)	(L_1 300)	(L_2 149)	(L_3 98)	(P 18)
S. caprea shaved plus salicin	94.2%	47.5%	38.1%	79.4%	12.5%
	(Ad 7)	(L_1 120)	(L_2 63)	(L_3 39)	(P 8)

salicin. During the prepupal stage the mortality of the larvae reared on *S. caprea* with or without salicin is high compared to that on *S. nigricans*. Among other possibilities this high mortality could be due to a nutritional deficiency in the larvae reared on *S. caprea*. Measures of relative growth rate and of consumption would clarify this point.

The survival of the pupa and the success of its eclosion seem to be linked to the presence of salicin. No data are available on the causes of pupal mortality in the absence of salicin. The number of pupae are too small for statistical analysis. We would however suggest that salicylaldehyde contained in the exuviae of the third instar (see below) may act as a bactericide (Pavan, 1953) and thus protect the pupa from infection. Pupation occurs in the soil and soil surrounding the pupa may have been impregnated with salicylaldehyde from the exuviae.

Growth to adult is slower on *S. caprea* (26 days) than on *S. nigricans* or *S. caprea* shaved, with or without salicin (24 days). This difference arises during the first instar (8 days instead of 6) when trichomes are present and is presumably due to food shortage.

The fresh weight of the beetles reared on *S. nigricans* (5.97 ± 0.68 mg, $n = 21$) is significantly higer ($P < 0.05$) than that of those reared on *S. caprea* shaved with salicin (5.49 ± 0.08 mg, $n = 13$) or without it (5.39 ± 0.74 mg, $n = 8$). The presence of salicin is not in itself enough to restore normal weight.

Larvae of *P. vitellinae* possess two pairs of dorsal thoracic glands and seven pairs of dorsal abdominal glands which are extruded when the larvae are disturbed, releasing droplets of secretion. Larvae bred on *S. nigricans* produced a copious amount of secretion shown by GC to contain salicylaldehyde. Larvae which developed on shaved *S. caprea* extruded their glands normally but no secretion could be collected. No odour was perceptible, and no trace of salicylaldehyde was detected by gas chromatography. When salicin was added to the shaved leaves of *S. caprea* the secretion was partly restored, but the volume was smaller and the con-

Table 3. Defensive secretions of the *Phratora vitellinae* larvae.

	Larval food		
	S. nigricans	*S. caprea* shaved	*S. caprea* shaved + salicin
Number of larvae	30	60	30
Volume of secretion (10^{-3} μl/insect)	84 ± 41	0	58 ± 22
Concentration of salicylaldehyde in the secretion (μg/μl)	684.5	0	162.0
Salicylaldehyde in the exuviae of 3rd instar larvae (μg/exuvia)	107.2	0	15.9

centration of salicylaldehyde lower than in the larvae fed with their normal host plant (Table 3). Thus salicylaldehyde appears to be derived from salicin, and possibly from related phenolglycosides, but the relationship between the concentration of salicin in the food, the volume of the defensive secretion and its concentration of salicylaldehyde remains to be investigated. The exuviae of the third instar larvae reared on *S. nigricans* or on *S. caprea* plus salicin also contained salicylaldehyde. There is no salicylaldehyde in the exuviae of third instar larvae reared on *S. caprea*.

Discussion

In nature, *P. vitellinae* selects *Salix* spp. containing phenolglycosides but no condensed tannins in the leaves. Salicin (and possibly other glycosides) are the precursors of the salicylaldehyde secreted for defence by the larvae. Several other chrysomelid larvae, all *Salix* or *Populus* feeders, also produce salicylaldehyde (Pasteels, 1982). *Chrysomela tremulae* produces labelled salicylaldehyde when fed labelled salicin (Pasteels et al., in prep.), thus corroborating Wain (1943) and Pavan (1953). Salicylaldehyde is secreted in aqueous solution and no secretion at all is produced when salicin is not present in the food. Salicin may thus be essential for the functioning of the glands.

Most chrysomelid larvae possessing similar glands feed on herbs (Cruciferae, Polygonaceae, Ranunculaceae) and secrete manufactured cyclopentaterpenoid. Their secretions differ qualitatively and quantitatively from species to species, or even between far distant populations of the same species (Pasteels, 1982). A few species feed on trees, nearly always the Salicaceae and rarely *Alnus* (Betulaceae). Only the larvae feeding on Salicaceae produce salicylaldehyde and this has occurred independently in at least two genera (*Chrysomela* and *Phratora*) belonging to two different tribes. This convergence contrasts with the diversity of the larval secretions of the herb-feeding species. Pasteels (1982) suggested that salicylaldehyde production is secondary in the evolution of chrysomelid defences, resulting from a shift from herbs to woody plants. Some *Salix* spp. may have been specially favourable for such a switch, not only because they contain secondary substances (e.g. salicin)

which provide precursors for cheap defensive secretion, but also because of the absence of condensed tannins. Such a tree may be more appropriate to an herbivore adapted to digesting herbs. A study of herbivore diversity on the full range of *Salix* and of feeding specificity would assist in evaluating this hypothesis and might provide insight into the anomalously large number of herbivores reported on *Salix* (Southwood, 1961).

Salicin is however not needed for food acceptance by *P. vitellinae*. The avoidance of *Salix* spp. devoid of phenolglycosides is mainly due to their hairiness, which is responsible for the high mortality of the larvae on the leaves of the *Salix* spp. containing no salicin. The importance of leaf pubescence has often been stressed (Johnson, 1975; Levin, 1973), and the suitability of the various *Salix* spp. for *P. vitellinae* seems to be clearly due to physical characteristics of the leaves. It is not excluded however that chemical factors may play a role in more subtle food preferences. *P. vitellinae* prefers the introduced American hybrid *Populus trichocarpa deltoides* to the native *P. nigra* (Finet & Gregoire, 1981). This preference seems to be based more on chemical than on physical differences between the spicies (Finet, pers. commun.), and both species contain salicin.

Larvae fed on *S. caprea* with or without salicin yielded lighter adults than those fed on *S. nigricans*. This difference might be due to the presence of condensed tannins in *S. caprea* leaves. Although there is much literature on the potential role of tannin in insect plant relationships (Bernays, 1981), very few quantitative studies have been done apart from Feeny (1970) and Wint (1982). We need to know more of their effect on the fitness of these chrysomelids.

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Sensory physiology of insect-plant relationships — round-table discussion

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This review of the roundtable discussion is not an exact recapitulation but more a personal view of the presented results supplemented with some recent data. Since, in our present view, insect-plant interactions are to a large extent based on chemical cues, chemoreception was the central theme of the discussion. We have tried to identify also major obstacles for progress in this field and suggest new approaches to the study of sensory physiological aspects of insect-plant relationships. The main topics of the discussion have been selected by the author and they will be treated in the same sequence. The first two chapters have been chosen to discuss methodological and technical detail of ongoing research; the remaining are intended to be of general interest.

Stimulating techniques

Stimulation of chemoreceptor sensilla is the method to study the responses of the sensory neurones with electrophysiological techniques. It is therefore crucial to know the possibilities and limitations of existing and new techniques.

Olfaction The reproducible stimulation of olfactory sensilla is relatively difficult (Schneider, 1968; Kafka, 1970). One of the still used solutions has been developed by Kafka (1970) and modified by Sass (1976). The odourants are dissolved in paraffin oil and this mixture (in dilution series) is used to saturate the air in a syringe. From the syringe odour samples (number of molecules per air volume determined by the partial gas pressure corresponding to the odourant concentration in the paraffin solution) can be delivered by a precise movement of the piston of the syringe. The disadvantage of this technique is that relative large amounts of pure compounds are needed and that the system has to be calibrated for each compound. Less sophisticated versions of this technique are not so accurate but have still proven to be useful (e.g. Ma & Visser, 1978; Guerin & Visser, 1980). A completely

different method has been developed by Arn et al. (1975) using the effluent of a capillary GLC as a stimulus delivery system. This technique allows both the use of complex mixtures and pure compounds as stimuli. Mixtures are separated into pure components and stimulate in successions. The compounds are quantified simultaneously by the FID signal fed by the split effluent from the column. The stimulation is both highly reproducible and definable in terms of quantity and quality. So far only EAG recordings have been performed using plant extracts and headspace odours (Guerin & Städler, p. 95). Recently van der Pers (unpubl.) succeeded to use the GLC technique to stimulate single sensilla. The main disadvantage of the technique is the time elapsing between injection and elution of the separated compounds from the column.

Contact chemoreception The easiest and probably also the most reliable technique to record from contact chemoreceptive sensilla is the tip recording method: a glass capillary containing the stimulating solution is used as the recording electrode acting as an electrolytic bridge linked to the amplifier. The stimulating compounds have to be therefore in a water phase. An alternative to this technique is the sidewall recording method allowing the recording of the nervous activity of the receptor cells independent of the stimulus application (for a detailed description of both methods see Dethier, 1976). Dethier (1972) used this technique to show the sensitivity of contact chemoreceptors of the blowfly to vapours of non-polar chemicals.

The first evidence for the perception of non-polar plant compounds by the contact chemoreceptor cells of the sensilla on the maxillary palp of the locust has been presented by Blaney (1975). Sidewall recording proved to be difficult and unreliable, therefore the author applied the tip recording method, using a diluted salt solution in which leaf surface waxes had been sonicated. Since this approach proved to be unsuitable for the stimulation of the contact chemoreceptor cells in the tarsal sensilla of the carrot fly (with tip recording) a series of other possibilities were investigated.

Reproducible results were obtained with methanol leaf surface extracts which have proven to be also active in the oviposition bioassay (Städler, 1977). As shown in Figure 1 a 10% methanolic salt solution produced a distinct reproducible response in the tarsal D-hairs of the carrot fly. In these recordings the cabbage leaf surface extracts seem to be indistinguishable from a pure methanolic solution (control). Based on the recorded 'normal' response to sucrose none of the different fly species tested (carrot, cabbage and fruit flies) showed any signs of a deleterious effect produced by the contact with 10% methanol. This is in agreement with the fact that the rejection threshold of 50% of blowflies and crickets for methanol is 5-10 mol/l (16-32%) (Dethier, 1951). Basically the same idea has been used earlier by Ma (1977) to dissolve the sesquiterpenoid Warburganal in a phosphate buffer with ethanol (final concentration in the stimulating solution 0.1%). These examples show that alcohols can be used to test compounds or extracts which cannot be dissolved in water by themselves.

An other promising technique for the stimulus application of contact chemoreceptors with non-polar compounds (fraction of tomato leaf extract) was described by de Boer (unpubl.). He used glassfiber wicks impregnated with the non-

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Sucrose 300mM   **1sec**   **1mV**

Methanol 10% (extract solvent)

Cabbage leaf surface extract

Carrot leaf surface extract

Cabbage leaf surface extract

Carrot leaf surface extract
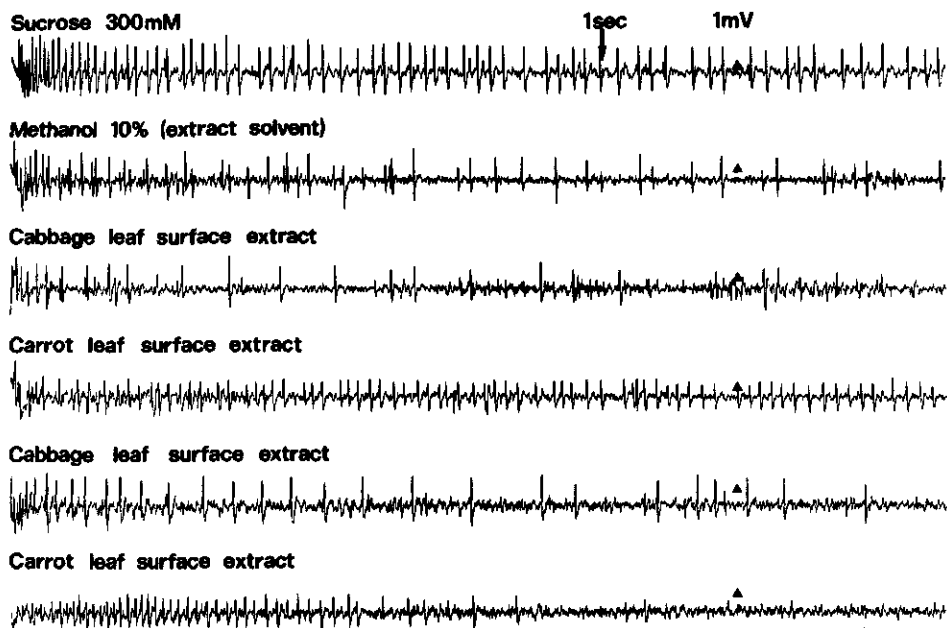
Fig. 1. Tip recordings from a D-hair of the last tarsal segment (prothorax leg) of the carrot fly (*Psila rosae*, Diptera: Psilidae). All stimuli contained 100 mmol/1 NaCl as an electrolyte. Sucrose at 300 mmol/1 was used as a standard stimulus at the beginning, during and at the end of the experiment as a control for possible deleterious effects of methanol. Time interval between the successive stimulations was at least 10 minutes (Städler, unpubl.). Calibration of recordings: duration after begin of stimulation: 1 second; amplitude: 1 mV.

polar fraction of the extract. After evaporation of the organic solvent the wick was wetted with a diluted salt solution which provided electric contact with a silver wire connected with the amplifier.

Analysis of electrophysiological recordings

Most chemoreceptor sensilla contain more than one receptor cell. Consequently more than one receptor neuron can be firing (producing action potentials, nerve impulses or spikes) in response to the stimulation (examples see Figure 1). Since the message to the central nervous system (CNS) of each individual receptor cell is contained in the time distribution of the nerve impulses, the complex multineuronal recording has to be analysed first by separating the different impulses and assigning each type to one chemoreceptor cell. Given the complexity and density of the information in the recorded spike trains the question arises if computers could be used for the analysis. Hanson gave a review of the technical possibilities and the different approaches taken by different investigators.

The first question is to find the best criteria to discriminate between the different impulses. In several laboratories the spike amplitude (positive to negative peak) is

used and believed to be sufficient. Others use in addition some measurement of the time course of the impulse such as the time difference between positive and negative peaks (e.g. van der Molen et al., 1978). A further elaboration has been introduced by O'Connell et al. (1973) using the standard deviation of 30 samples of each impulse from the mean voltage. These authors found that this criterium had a separation effectiveness of 97-98% compared with an experienced observer. Other criteria such as spike amplitude were significantly less effective. Probably the most sophisticated techniques are used at present by Piesch & Wieczorek (1982) and by Hanson and his coworkers.

Piesch & Wieczorek use a program working with a trainable pattern classifier whereby 'safe' candidate spikes (amplitude histograms) are chosen to form a set of training data based on four features of each spike candidate. Classification of all spikes is performed based on the training data with optimum decision surfaces in a multidimensional space.

Hanson et al. (unpubl.) are generating prior to the actual analysis a template of a particular spike and are classifying the impulses according to variation (variation threshold for example 20%) from the templates. The examples presented by Frazier and Hanson showed that the computer analysis of multineural recordings is possible and that in some cases impulse amplitude alone is not a good enough criterion for the discrimination of different cells (Figure 2).

The recognition of individual chemoreceptor cells by means of spike characteristics should ideally be followed by a physiological characterization, provided the qualitative and quantitative composition of natural stimuli (host and non-host plants) are known and the compounds are available. The sensitivity spectra of the individual receptor cells can be determined (recent example Selzer, 1981). If spikes of different cells in a record cannot be differentiated clearly, by techniques mentioned, the sensitivity spectra and/or selective adaptation (cross adaptation) are the 'classical' approaches to decide how many and what kind of cells do occur (for recent examples see Wieczorek, 1976; Sass, 1978).

When the individual spikes of the different cells have been identified by the computer or the human observer the time interval distribution of the impulses can be analysed. The meaning of this coded information is read by the CNS and after recognition may result in adapted behaviour like orientation, biting, feeding or oviposition. The spike train which is divided usually in a phasic and tonic portion can be quantified with different measurements such as spike frequency in a preset period, maximal frequency, time of the maximal frequency, adaptation rate, frequency in the tonic portion of the spike train (see van der Molen et al., 1978). Dethier & Crnjar (1982) compared the actual distribution of the interspike intervals produced by stimuli (plant saps). Further these authors tested also for serial dependence in the intervals by comparing the autocorrelations of normal and shuffled data. Using these analyses they were able to show the existence of sensory codes in the spike trains obtained from the sensilla styloconica of the larvae of *Manduca sexta* in response to plant saps.

A detailed advise for the use of automated devices or manual methods for spike train analysis seems at present and probably also in the future not possible because: (1) a systematic comparison of discrimination parameters and analytical methods

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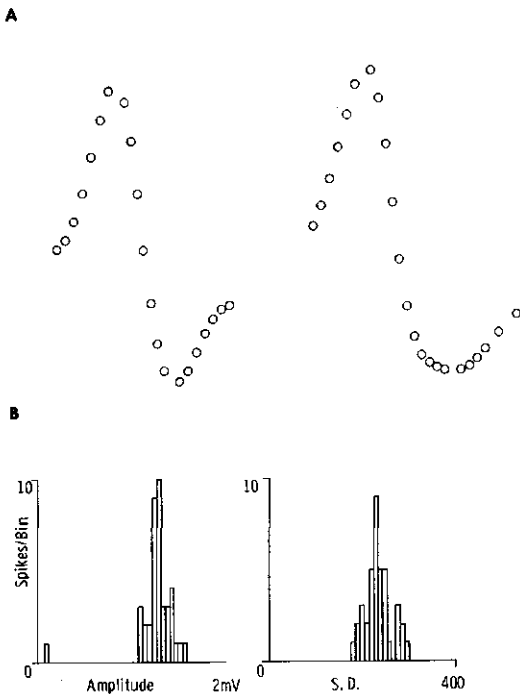
Fig. 2. A. Digitized action potentials of the salt cell (left) and sugar cell (right) of the tarsal chemoreceptors of the housefly. B. Histograms of spike parameters of a response to salt-sugar mixture. Amplitude histogram does not resolve the two populations of spikes as well as does standard deviation (Frazier & Hanson, unpubl.).

have not been performed and because, (2) the preparations (insects, impedance of the sensilla), the stimuli and equipments are so different. However, there can be no doubt that many promising techniques and analytical methods are presently available which will help future studies significantly.

Correlations between sensory physiology and behaviour

The experimenter has several possibilities to try to understand the sensory message coded in the time distribution of impulses to the CNS. The most direct attempt to understand the deciphering system would be the recording from central interneurons.

This approach has so far only been applied in one phytophagous insect, the locust, by Boeckh (1973, 1974). In the deutocerebrum of this insect neurones were identified reacting with immediate or delayed excitation, inhibition or off-responses to the stimulation of the antennal receptors. Comparing the sensitivity spectra of the different olfactory cells with that of the central neurones Boeckh (1974) concluded that (1) the sensory input from the sensilla basiconica is more important than that of the s. coeloconica and that (2) the receptor cells with different odour specificities

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converge onto central neurones with varying sensitivity spectra. This conclusion was further supported by the fact that the inhibition of the central neurones was apparently caused by an excitation of receptor cells rather than an inhibition. Boeckh (1974) could at that time not correlate the observed sensitivity of the central neurones to grass odour with that of pure compounds which are constituents of this complex odour mixture. Unfortunately these promising results have so far not yet been extended in the locust or any other phytophagous insect. However, the successful investigation of the central neurones of the sensory pathways in the cockroach and other insects in response to food odours and pheromones do show that the study of the CNS is difficult but very promising (Boeckh, 1980; Schneider & coworkers, unpubl.).

The method so far applied in most investigations to elucidate the coding of sensory qualities and quantities has been to correlate the responses of chemoreceptor cells with (1) the stimulus, namely its chemistry (quality) and its concentration (quantity) and/or (2) the behavioural reaction (qualitatively described or quantified) released by the same stimulus. If we attempt to understand the code meaningful for the CNS and the animal, correlating the sensory input to the CNS with its output (adapted behaviour) seems to be the obvious approach. However, we should be aware of the fact that correlations do not prove causative relations and therefore they cannot replace future studies of the CNS and its decoding mechanisms.

Since the last review of the subject (Städler, 1980) further advances in the understanding of sensory coding of both olfactory (Selzer, 1979, 1981; Visser, 1979) and contact chemoreceptors (Blaney, 1981; Blaney & Winstanley, 1980; Clark, 1981; Dethier & Crnjar, 1982; van Drongelen, 1979, 1980) have been made. These studies and some not yet published data presented in the discussion do support the view explained by Dethier (see also Boeckh, 1980; Dethier & Crnjar, 1982; Schoonhoven, 1981) that basically three types of coding can be distinguished today.

Labeled lines The characteristics of labeled lines are highly specific receptor cells with narrow sensitivity spectra which are not overlapping (at natural concentrations) with other cells. Classical representatives of such cells are the glucosinolate receptors of insects associated with Cruciferae and a few other examples mentioned in earlier reviews (Städler, 1980).

Temporal patterns The first evidence for such coding (different latencies, rates of adaptation and after-effects) has been observed in olfactory receptor cells of a Lepidoptera larvae by Dethier & Schoonhoven (1969). Mitchell (unpubl.) presented evidence that solanum alkaloids generate a special type of latency which may be correlated to the deterrency of these compounds. Recently Dethier & Crnjar (1982) found also stimulus specific time patterns in the response of one of the contact chemoreceptor cells of *Manduca sexta*.

Across-fibre patterns In contrast to the labeled line (cell) coding the specificity of the receptor cells producing across-fibre patterns is much lower and the sensitivity spectra are overlapping. The ensemble of the responding receptor cells produce a stimulus specific pattern which can be expressed as the ratios of spike frequencies

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between the different cells. This type of coding seems to be the evolutionary solution to the problem of receiving highly complex natural mixtures of different compounds with a limited set of receptor cells. An increasing number of publications gives evidence for the existence of across-fibre patterns in the sensory coding of chemoreceptors.

A major difficulty in the study of sensory coding has always been its variability. Many sources of this variability in addition to the genetic determination which will be discussed later, have been identified. The recognition of the components of this variability has led to improved designs of electrophysiological experiments (e.g. Dethier & Crnjar, 1982). Van der Molen et al. (1982) recently quantified three sources of variability in the blowfly and found that the main variance is due to the individual insect. Blaney (1981) studying sensory coding in the sensilla of the maxillary palp of the locust quantified the variance between the sensilla of one individual palp. He showed, using an analysis of variance, that the variance between both the sensilla and the different solutions which are discriminated by the animal is highly significant. Furthermore the interaction between the sensilla and the solutions was also statistically significant thus proving that the different sensilla do respond differently to the test solutions. This is a clear indication for the existence of across-fibre (sensilla) patterns.

In order to study sensory coding and across-fibre patterns it is therefore important that (1) the variability between sensilla has to be considered as well as the individual variability and that, (2) across-fibre patterns may not only exist between the cells of one sensillum but also between the sensilla of one sensory organ. This points also to our limitation in our ability to record from many sensilla and different sensory organs at the same time. In fact we will never be able to record all the sensory input simultaneously that the CNS of the insect is able to use for its recognition tasks.

A special question of coding in across-fibre pattern is the distinction between quality and quantity of the stimulants. Since Dethier & Crnjar (1982) found no significant difference between normal and diluted (25%) plant saps it seems at least in *Manduca* that the main information content is quality (type of plant) and that quantity is of minor importance. This may be brought about by the fact that the receptor cells are already saturated by the normal and diluted plant sap and can therefore not react to the 4-fold change of concentration.

Correlation between sensory coding and the perceived environment

It has always been tempting to believe that the type of feeding specialization should be paralleled by corresponding specialization in chemoreception. Dethier & Kuch (1971) and Dethier (1973) tested this assumption by comparing the sensitivity of mono-, oligo- and polyphagous caterpillars of related and unrelated species to pure chemicals and to plant saps of host and non-host plants. They found that there is no correlation between life style (feeding strategy) and sensory perception in the contact chemoreceptors of Lepidoptera larvae. In this sense the authors found no unique sensory patterns for acceptance (food plants) nor rejection (non-host plants). This was true for the monophagous as well as for the polyphagous species. Since

each plant independent of its acceptability was generating a different sensory pattern, Dethier (1973) concluded that caterpillars do perceive different plant species as qualitatively different.

These conclusions seem at least partially to be in contrast with the existence of receptor cells specifically sensitive to 'host plant sign stimuli' mediating feeding stimulation as discovered by Schoonhoven (1967), Ma (1972 in lit.) and Blom (1978) in *Pieris brassicae* and recently by Schoonhoven et al. (1977) and van Drongelen (1979, 1980) in some *Yponomeuta* caterpillars. Although these studies do not imply that labeled line coding alone could explain host plant selection in these species, it seems likely that they represent dominant features of across-fibre patterns. This hypothesis needs however to be tested. A possible explanation why Dethier was not able to identify labeled line coding in his investigation has been suggested by Dethier (1973): Some of the most typical host plant compounds were unfortunately not available for this comparative study. But the fact remains that the author found no signs of labeled line coding in the response to the various host plants.

Since leaf surfaces are presumably chemically less complex stimuli than leaf saps, Städler (1980) proposed that sensory coding in the sensilla involved in their perception is not so complex. The recordings in Figure 1 seem to support this view because one receptor cell of the tarsal sensillum of the carrot fly seems to respond to the extract of the carrot leaf surface. Since the corresponding sensilla of the cabbage fly do not respond to the extract it is unlikely that the recorded response of the carrot fly is accidental.

Given the existence of a remarkable interindividual variability it is not surprising that all comparative studies of related and unrelated species show drastic differences (recent references Blaney, 1981; van Drongelen, 1979). The genetic determination of sensory function has been further substantiated by recent investigations. Wieczorek (1976) found two strains of *Mamestra brassicae* with a distinctly different sensitivity to glucosinolates. In addition Schoonhoven (unpubl.) reported that a systematic selection of salicin-sensitive (behavioural assay) individuals of *Manduca sexta* led to a strain which has a receptor cell very sensitive to this compound. The genetics of chemoreception in phytophagous insects is a new highly interesting aspect of the sensory physiology of insect-plant relationships as has been proved by van Drongelen & van Loon (1980). These authors crossed two closely related *Yponomeuta* species with a different sensitivity for dulcitol, phloridzin and prunasin. In the crossed animals the sensitivity to dulcitol and prunasin proved to be dominant, that for phloridzin showed an intermediate expression.

Sensory physiology of insect-plant relationships in the future

In the last symposium on insect-plant relations Dethier (1978) addressed already this question. Despite the mentioned progress made in the last years it seems that our problems and aims have changed little. Therefore it is appropriate to recall the conclusions made by Dethier (1978) summarized in the three desiderata for future electrophysiological investigations:
— more detailed knowledge of the action (specificity) spectra of receptor cells

– further analysis of the effect of mixtures (natural stimuli) on sensory systems
– investigation of the central nervous system

Dethier (1978) identified also our main problem inhibiting progress of sensory physiology: (cit.) 'The greatest obstacle to advance here, and indeed to any progress in understanding receptor sensitivity, neural coding, central nervous system interpretation of incoming messages, nutritional adequacy, and detoxifying mechanisms is the paucity of information regarding plant chemistry and physiology, especially that of the living plant as opposed to static samples of plant tissue'. Several authors have realized this need to study the qualitative and quantitative chemistry of the living plant in relation to behaviour and sensory physiology (Guerin & Städler, p.95; Selzer, 1981; Städler & Buser, p.403; Thibout, p.107; Visser, 1979; Woodhead et al., 1980, 1982). Undoubtedly these and future studies will help to understand the sensory physiology of insect-plant relations.

The question arises if sensory physiology could directly increase our understanding of plant chemistry in relation to host plant selection. As pointed out already in the first section one such approach is the use of a combination of GLC with electrophysiological recordings for the analysis of volatiles emanating from intact (or disrupted) plants. In this regard the simpler but effective approach to collect samples of GLC or HPLC columns used by Selzer (1981) should not be disregarded. To our knowledge fractions of a chemical separation of non-volatile plant compounds have so far not yet been used to stimulate contact chemoreceptors. But Prokopy et al. (1982) demonstrated successfully the use of this approach to identify the female gland which is the site of production of the oviposition-deterring pheromone in *Rhagoletis pomonella*.

Obviously the use of electrophysiological recordings to monitor the 'activity' of plant extracts and fractions has also its limitations such as: (I) electrophysiological recordings have to be calibrated using behavioural observations, and (II) controls for inactive sensilla have to be included. These difficulties should not distract from possible advantages: (1) stimulations of single sensilla can be performed with very small samples, (2) some receptors must be sensitive to minor components or synergists not active in a particular behavioural assay, and (3) very small concentrations of specific compounds may be detected which would escape a standard chemical analysis using GLC in combination with a flame ionization detector (see Guerin & Städler, p. 95).

Some of these ideas about the future of sensory physiology and the possible approaches to investigate insect-plant relations are in progress in several laboratories. It can be hoped that the accumulation of more detailed knowledge of the plant compounds relevant to host plant selection will allow a significant progress in the understanding of the sensory physiology of insect-plant relationships and its relevance for the behaviour and ecology of insects.

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Behaviour

Host odour perception in three phytophagous Diptera — A comparative study

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Abstract

A high degree of olfactory specificity was observed by means of the electroantennogram (EAG) in the carrot fly and onion fly, respectively, for the host-related volatiles trans-asarone (2,4,5-trimethoxy-1-propenyl-benzene) and propyldisulphide; the cabbage root fly, by contrast, showed less specificity for allylisothiocyanate with an optimal EAG response to dimethyldisulphide. While the carrot fly is selectively tuned to the perception of leaf aldehydes and the onion fly to leaf alcohols, the cabbage root fly olfactory receptors demonstrated no selectivity to a single functional type of green leaf volatile. Aliphatic aldehydes of between 7 and 9 C atoms evoked the highest EAG responses in all three oligophagous species. Their sensitivity spectra to individual volatiles were reflected in EAG responses to headspace vapours over host and nonhost foliage. Combined gas chromatography and electroantennography indicated that the carrot fly was 10^4 times more sensitive to host-related propenylbenzenes than to more generally occurring volatiles in the headspace over carrot foliage.

Key-words: chemoreception, olfactory receptors, plant odours, *Psila rosae, Delia brassicae, Delia antiqua*

The specificity of the behavioural response exemplified by oligophagous insect species for odours typifying their host plants suggests some specialized tuning of their olfactory receptors located in the antennae. Pertinent examples include the response of the carrot fly *Psila rosae* F., to propenylbenzenes in carrot *Daucus carota* L. (Berüter & Städler, 1971; Städler, 1972; Guerin & Städler, 1980), the cabbage root fly *Delia brassicae* Wied., to isothiocyanates in Cruciferae (Wallbank & Wheatley, 1979; Finch & Skinner, 1982) and the onion fly *Delia antiqua* Meigen, to sulphur compounds in onion *Allium cepa* L. (Matsumoto & Thorsteinson, 1968; Ishikawa et al., 1978; Pierce et al., 1978; Didonis & Miller, 1981). By contrast, no host specific compounds have been identified in the Solanaceae which mediate in host selection by the Colorado beetle *Leptinotarsa decemlineata* Say (Visser, 1979a). Instead, evidence has been presented indicating that this oligophague responds to a specific blend of generally occurring green leaf volatiles released by potato (Visser & Avé, 1978; Visser et al., 1979).

We tested the hypothesis that the antennal receptors of three oligophagous Diptera, the carrot fly, cabbage root fly and onion fly, show a specialized sensitivity to their respective host plant volatiles by comparing their electroantennogram (EAG) responses to:

— known host specific compounds influencing host plant selection
— a group of generally occurring green leaf volatiles
— vapour samples (headspace odours) over intact foliage of the three host plants.

By employing combined gas chromatography (GC) and EAG analysis of a headspace sample from carrot foliage we compared the relative activity of the different constituents.

Materials and methods

The EAG recording system, inclusive of antennal preparation and odour delivery, was essentially the same as that previously described (Guerin & Visser, 1980). Plant volatiles purchased from commercial sources were screened at concentrations of 100 μl/ml and 1 μl/ml in paraffin oil on four female antennae of each of the three species; all compounds were $\geqslant 95\%$ pure (GC). While most compounds evoke a sizable EAG response at 100 μl/ml (2.5 mg source) the number perceived at 1 μl/ml (25 μg source) is markedly reduced. For this reason only the responses recorded at the latter level, which facilitate the establishment of true differences between the volatile stimuli, are accounted for here. Due to the marked decline in the absolute response of an individual antenna during the experimental period, the amplitude of the EAG evoked by an individual stimulus is expressed relative to a standard (cis-3-hexen-1-ol) applied at regular intervals.

The vapour over foliar samples of carrot, Brussels sprouts *Brassica oleracea* var. *gemmifera* Zenk., and leek *Allium porrum* L., was sampled by placing some 60 g of foliage (stems in water) from each of the plant species in three sealed glass cylinders of 5 l. After 24 h a 1-ml sample of air was taken through a septum in the lid of the container and injected into the air stream passing over the antenna. New foliage samples were prepared on four consecutive occasions permitting a comparison of the responses evoked by the host vapour with that of the non-hosts on four female antennae from each of the three species.

The differential response of the carrot fly antenna to the constituents of the headspace over carrot was recorded by GC analysis of the vapour sample on an SP-1000 glass capillary column (15 m × 0.3 mm i.d.) with an outlet splitter connected to a flame ionization detector (FID) and electroantennographic detector (EAD; Arn et al., 1975). Sufficient material to obtain a GC profile of the volatiles over the foliage was obtained by shredding 75 g of leaf material into a 1-l glass bottle. Within an hour a 0.5-ml vapour sample was taken in a gas-tight syringe through a septum in the lid and injected (splitless mode) onto the capillary column. The resolved constituents of the headspace vapour passed simultaneously, via the effluent splitter, over the FID and EAD detectors (split ratio 2:1); details of the EAD detector will be described elsewhere (Guerin et al., in prep.).

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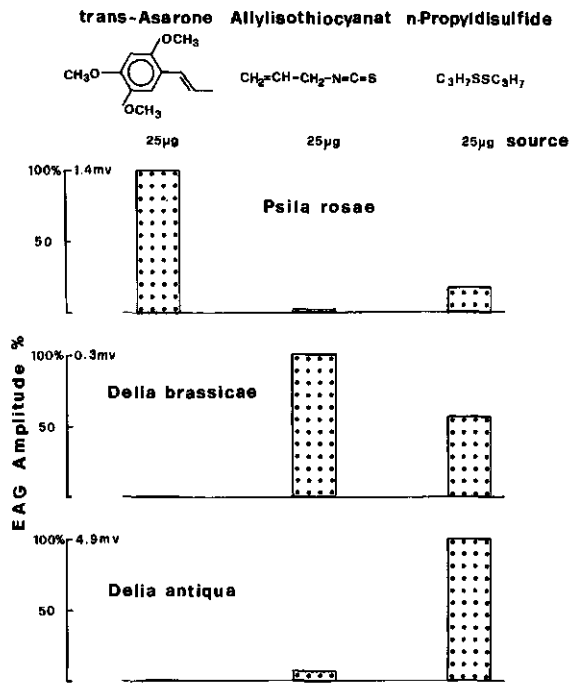
Fig. 1. EAG responses of three phytophagous Diptera to plant volatiles expressed as a percentage of the response to the respective host plant attractant ($n = 4$).

Results and discussion

Specificity to host-related volatiles   The manner in which the olfactory receptors of the three species are specifically tuned to the perception of 'relevant' information is demonstrated in Figure 1. The carrot fly antenna is specific in so far as the host-related propenylbenzene trans-asarone (2,4,5-trimethoxy-1-propenylbenzene) evokes a strong EAG response while the *Brassica* and *Allium* related products evoke little or none. Trans-asarone evoked the strongest response of over 80 carrot volatiles screened for EAG activity in the carrot fly (Guerin et al., in prep.); it is present in the cuticular wax of carrot foliage and acts as an oviposition stimulant (Städler & Buser, p.403) and is attractive in the field, the number of flies captured being linearly related to the quantity applied per trap (Guerin & Städler, 1980). Since the product is also present in the headspace vapour of carrot foliage (peak b, Figure 5) this indicates its role in host plant selection by the carrot fly.

The cabbage root fly was found to be insensitive to trans-asarone and sensitive to its host specific glucosinolate hydrolysis product allylisothiocyanate (Figure 1), which together with the non-volatile glucosinolates is an oviposition stimulant (Traynier, 1965; Zohren, 1968; Nair & McEwen, 1976) and field attractant for the fly (Finch & Skinner, 1974). Propyldisulphide, an onion fly attractant, evoked a surprisingly high EAG response amounting to over 50% that of allylisothiocyanate.

This is plausible since the chemically related dimethyldisulphide is present in the headspace over cauliflower *Brassica oleracea* var. *botrytis* L. (Wallbank & Wheatley, 1976), and evoked an EAG response significantly higher than that of allylisothiocyanate (Table 1). This may indicate that the cabbage root fly is not responding to one type of host volatile but presumably to a blend. The field data of Finch & Skinner (1982) indicate that this may be the case since traps loaded with mg amounts of volatile host plant extracts are as effective as those with g quantities of allylisothiocyanate.

Propyldisulphide evoked the strongest EAG response in the onion fly (Table 1); this species is apparently insensitive to trans-asarone and responds weakly to allylisothiocyanate (Figure 1). Propyldisulphide is an oviposition stimulant (Matsumoto & Thorsteinson, 1968) and field attractant for the onion fly (Matsumoto, 1970) and a major constituent of the headspace over onion (Ishikawa et al., 1978; Pierce et al., 1978; Ikeshoji et al., 1980).

Some selective sensitivity of the olfactory receptors was observed for the perception of the more host specific volatiles in the onion fly. Propyl-sulphides were more stimulatory than any of the other volatiles tested and disulphides with either propyl- or methyl-moieties were more active than their monosulphide analogues (Table 1). This is in accordance with observations of Vernon et al. (1978) and Ishikawa et al. (1978), respectively, based on oviposition responses, indicating that a thiopropyl function, among others, was responsible for olfactory stimulation in the onion fly and that two sulphur atoms are necessary for optimal activity. Some discrepancies were found between the EAG data (Table 1) and the oviposition studies cited above. In the EAG, dipropylsulphide is in the order of 6 to 7 times as active as methylpropylsulphide despite the oviposition response to their disulphide analogues where methylpropyldisulphide is 4 times as active as propyldisulphide (Pierce et al.,

Table 1. EAG responses[1] of *Delia brassicae* and *Delia antiqua* to *Brassica* and *Allium* related volatiles at 1 μl/ml as a percentage of the response to cis-3-hexen-1-ol at 10 μl/ml. (concentration of the compound in paraffin oil).

Compound	*Delia brassicae*	*Delia antiqua*
Propyldisulphide	9.6 ± 3.6	170.3 ± 18.4
Onion essential oil	60.7 ± 12.7	142.8 ± 15.8
Dipropylsulphide	12.4 ± 10.2	121.0 ± 15.9
Dimethyldisulphide	83.6 ± 37.3	47.3 ± 8.4
Propylmercaptan	3.1 ± 1.9	38.5 ± 4.6
Methylpropylsulphide	27.5 ± 13.6	18.8 ± 4.8
Allicin	0	0
Dimethylsulphide	3.1 ± 1.9	0
Allylcyanid	4.3 ± 3.0	0
Allylisothiocyanate	17.3 ± 6.0	12.0 ± 4.1
Tetrabutylisothiocyanate	11.3 ± 5.3	9.3 ± 3.5
Benzylisothiocyanate	2.7 ± 2.7	4.9 ± 2.9

1. Mean ± standard error ($n = 4$).

1978). Furthermore, propyldisulphide and propylmercaptan show EAG responses of 170% and 38% of the standard respectively, but differences of the same order of magnitude were not observed in oviposition studies (Matsumoto & Thorsteinson, 1968; Ishikawa et al., 1978; Vernon et al., 1978). These differences are not surprising in view of the lack of correlation between behaviour and EAG activity for onion volatile mixtures observed by Ikeshoji et al. (1981).

Green leaf volatile perception   Host odour perception most probably involves the concerted action of many sensilla to an array of volatiles released by the plant; it being the unique total pattern of the response which signifies 'carrot', 'cabbage' or 'onion' for the respective species. An underlying assumption of this theory is the capacity of the insect's olfactory system to discriminate between the qualities of plant volatile complexes. Given the widespread occurrence of 'green leaf' volatiles and their demonstrated role in odour quality coding by the Colorado beetle (Ma & Visser, 1978) it is of interest to compare the sensitivity spectra of the three phytophagous Diptera to these volatiles.

The olfactory system of the carrot fly is selectively tuned to the perception of green leaf aldehydes (Guerin & Visser, 1980). The functional significance of this adaptation has been established since leaf aldehydes are the predominant green leaf volatiles of carrot foliage and are the only components of the complex which attract carrot flies in the field (Guerin et al., in prep.).

Of the C_6 alcohols, aldehydes and esters screened for EAG activity in the cabbage root fly the highest responses were recorded for trans-2-hexen-1-al, cis-3-hexenyl acetate, hexyl acetate, hexanal and cis-3-hexen-1-ol, respectively (Table 2). The olfactory receptors of this insect are seen to be selective for 'relevant' components of

Table 2. EAG responses[1] of *Delia brassicae* and *Delia antiqua* to generally occurring green leaf alcohols, aldehydes and esters and their isomers at 1 μl/ml as a percentage of the response to cis-3-hexen-1-ol at 10 μl/ml (concentration of the compound in paraffin oil).

Compound	*Delia brassicae*	*Delia antiqua*
Hexanol	11.4 ± 3.2	71.8 ± 4.3
Heptanol	30.6 ± 8.1	82.0 ± 4.5
Trans-2-hexen-1-ol	10.9 ± 2.9	40.6 ± 3.5
Cis-2-hexen-1-ol	7.0 ± 1.5	27.4 ± 1.8
Trans-3-hexen-1-ol	9.6 ± 3.8	56.0 ± 3.3
Cis-3-hexen-1-ol	16.8 ± 1.8	25.1 ± 4.5
Hexanal	16.9 ± 9.9	9.2 ± 3.7
Heptanal	14.0 ± 3.4	63.4 ± 4.4
Trans-2-hexen-1-al	37.7 ± 6.2	25.6 ± 4.1
Hexyl acetate	17.5 ± 2.8	14.8 ± 2.3
Cis-3-hexenyl acetate	22.2 ± 8.4	13.7 ± 1.7

1. Mean ± standard error ($n = 4$).

the green leaf volatile complex since cis-3-hexen-1-ol and trans-2-hexen-1-al are important volatiles of cabbage, *Brassica oleracea* L. (Mac Leod & Pikk, 1979) and Cruciferae in general (Cole, 1980). The EAG active ester, cis-3-hexenyl acetate, predominates in the headspace over disrupted foliage of cauliflower, turnip, radish and wallflower (Wallbank & Wheatley, 1976). However, no single functional type of green leaf volatile acts as a predominant EAG stimulant for the cabbage root fly.

The most clear example of selective tuning was observed in the onion fly. The leaf alcohols, hexanol and heptanol elicit significantly higher EAG responses than the corresponding aldehydes, hexanal and heptanal (Table 2). Among the monoenic C_6 leaf alcohols the trans isomers evoke significantly higher responses than the corresponding cis forms. These observations are consistent with the finding that green leaf components of the headspace over leek are predominantly alcohols; hexanol figures as the most prominent saturated alcohol and together with the trans forms of 2-hexen-1-ol and 3-hexen-1-ol exercise a strong influence on the natural aroma of leek (Schreyen et al., 1976).

All three species show a high degree of conformity with respect to chain length in their responses to the aliphatic aldehyde series pentanal through to dodecanal, with each showing a peak in EAG activity for aldehydes of between 7 and 9 C atoms (Figure 2). This contrasts with optimal responses of the oak leaf weevil *Rhynchaenus quercus* L. (Kozlowski & Visser, 1981) and the Colorado beetle (Visser, 1979b) to saturated leaf alcohols and aldehydes with 6 C atoms, but is comparable with the
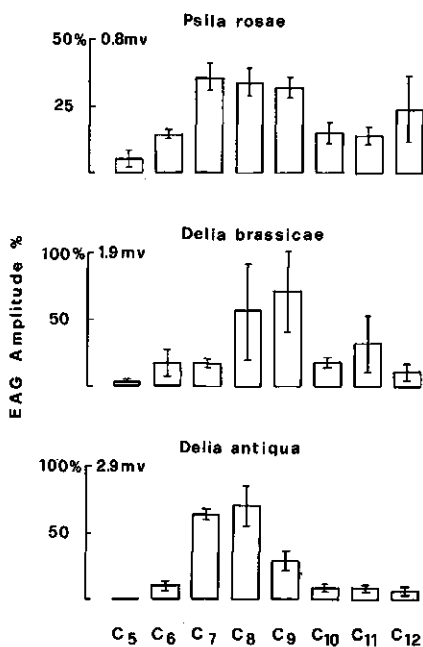
Fig. 2. EAG responses of three phytophagous Diptera to saturated aldehydes at 1 μl/ml (± standard error; $n = 4$) as a percentage of the response to cis-3-hexen-1-ol at 10 μl/ml (concentration of the compound in paraffin oil).

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DAUCUS BRASSICA ALLIUM
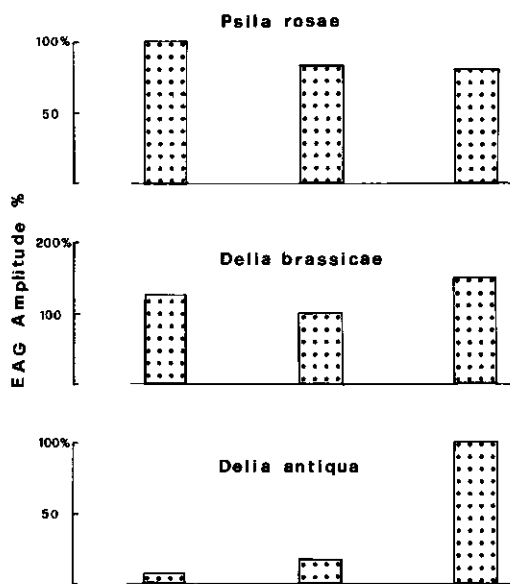
Fig. 3. EAG responses of three phytophagous Diptera to a 1-ml vapour sample over foliage of carrot, Brussels sprouts and leek expressed as percentage of the response to the respective host vapour ($n = 4$).

EAG responses of females of *Adoxophyes orana* and seven *Yponomeuta* species, where aliphatic alcohols and aldehydes are apparently maximal at a chain length of 7 (van der Pers, 1981).

Our results show that the olfactory organs of the three species are truly selective, permitting the insects to decipher some specific information regarding their host plants by means of some of the most generally occurring plant volatiles. In the case of the carrot fly we have obtained behavioural data in support of the claim that green leaf voltiles influence host plant orientation. Similar studies on the cabbage root fly and onion fly are required.

Headspace vapours　In a comparison of the EAG responses evoked by vapour samples over the intact foliage of carrot, Brussels sprouts and leek, the onion fly is most selective with values of 8.3% for carrot and 16.7% for Brussels sprouts, that of the response shown to leek (Figure 3). While the carrot fly shows a consistently higher response to its host odour the margin is low with Brussels sprouts at 80% and leek at 83% that of the response to carrot. However, in the company of root material, the amplitude of the carrot fly response to carrot is significantly higher as compared with cabbage (21%) and onion (17%) (Figure 4). This is reasonable since the root of carrot is the most odoriferous part of the host plant. By contrast, vapours over carrot

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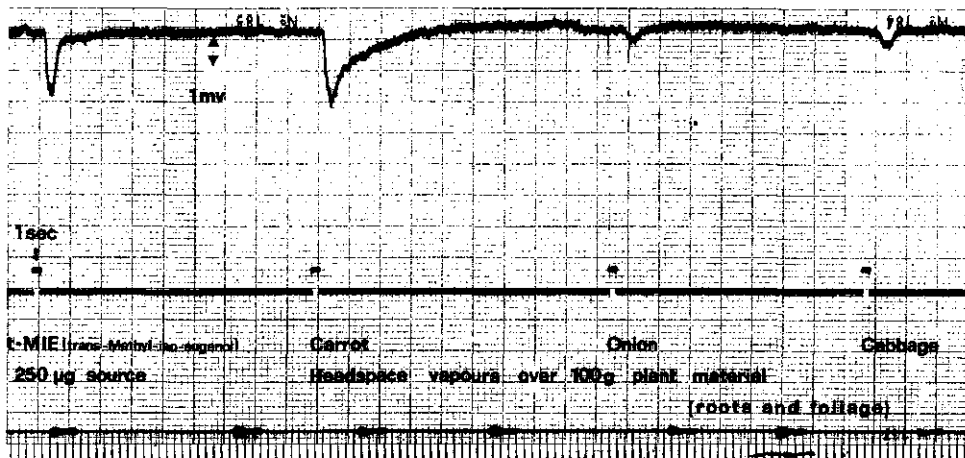
Fig. 4. EAG responses of a female carrot fly to 1-ml vapour samples over carrot, onion and cabbage.

and leek evoke higher EAG responses, 125% and 150% respectively, than that over Brussels sprouts in the cabbage root fly (Figure 3). The magnitude of the response to leek is to be expected since the cabbage root fly is more sensitively tuned to the perception on sulphides than the more host specific allylisothiocyanates (Table 1).

The absence of true differences in carrot and cabbage root fly responses to foliar volatiles may suggest that the host and non-host vapours are equally effective due to some common constituents. However, this finding does not argue against host plant selection on the basis of olfactory cues. Plant extracts and headspace vapour samples consist of mixtures of volatiles which may evoke very different EAG responses when applied singly (Figure 1 and Table 2) and may even contain compounds which are repellent to the fly. A high sensitivity to the latter products could explain the lack of fit. Clearly, differences in the composition of the vapour samples for which the insect is equipped to discriminate are not reflected in the EAG.

GC-EAD analysis of foliar volatiles from carrot   In the present study we employed GC-EAD to record the differential response of the carrot fly antenna to constituents of the vapour over shredded carrot foliage. By injecting the vapour sample directly onto the capillary GC column we avoid the use of a solvent whose tailing peak normally masks those of the lower boiling point constituents of the sample. The GC profile of the vapour may be divided into three zones (Figure 5):
— monoterpenes and leaf aldehydes eluting between 30 and 60°C
— sesquiterpenes and other products between 100 and 130 °C
— higher boiling point components eluting above 165°C

The corresponding EAD profile is remarkable in that no response was evoked by large amounts of commonly occurring compounds at the beginning of the chromatogram while the strongest EAD responses (relative to GC peak height) were generated at the end of the chromatogram by small quantities of the more host spe-

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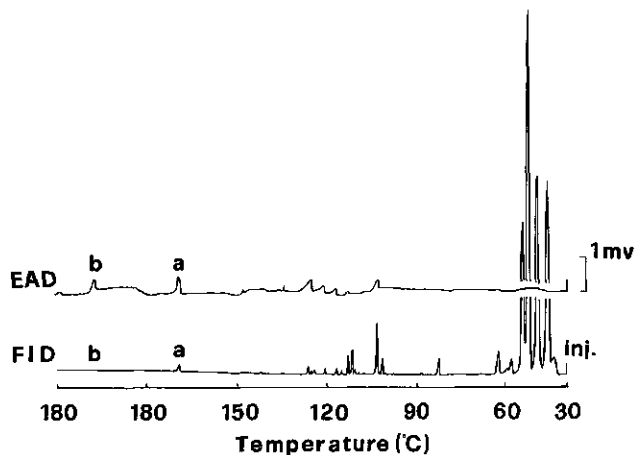
Fig. 5. Gas chromatograms with EAD response of *Psila rosae* female antenna and FID response to a 0.5-ml headspace vapour sample over shredded carrot foliage. Splitless injection, 1 min at 30°C, 10°C/min to 60°C and 5°C/min to 180°C with He as carrier gas. a: trans-methylisoeugenol; b: trans-asarone.

cific propenylbenzenes identified as trans-methylisoeugenol (3,4-dimethoxy-1-propenylbenzene; peak a) and trans-asarone (peak b) (Guerin et al., in prep.); note that trans-asarone was present in such low amounts as to be undetectable by the FID (Figure 5). In comparison to the relatively large amounts (between 50 and 100 ng each) of low boiling point components eluting between 30 and 60°C, the propenylbenzenes were estimated to be present at levels of 1 ng (a) and 10 pg (b) in the 0.5-ml vapour sample. The antenna of the carrot fly is accordingly equipped with receptors sensitively tuned to the perception of small amounts of more host specific low vapour pressure compounds and, apparently, relatively insensitive to more volatile generally occurring components present at levels 10^1 to 10^4 times higher in the headspace over shredded host plant foliage.

Conclusion

Our results show that the antennal olfactory receptors of each of the three Diptera are specifically sensitive to typical host plant volatiles. Even in the case of the generally occurring green leaf volatiles each species proved to be different and especially sensitive to components known to occur at higher concentrations in the headspace of host plants. Specificity for host plant compounds is, however, not absolute in the sense that each species is also capable of perceiving the odours of non-hosts. This is in accordance with the view that a phytophagous insect is capable of perceiving its own bouquet of compounds typifying each plant. However, not all components of these 'chemical images' are of equal importance, the specific host plant volatiles to which the insect is especially sensitive being most prominent.

Combined GC and electrophysiological recordings, as employed here on the car-

rot fly, seem to hold good promise for qualitative and quantitative analysis of plant compounds involved in host plant selection. If such studies can be combined with an analysis of the behaviour sequences involved, we can hope for a better understanding of host selection and its possible manipulation.

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Host plant chemicals responsible for attraction and oviposition in Acrolepiopsis assectella

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Abstract

The oviposition behaviour of the leek moth, a phytophagous specialist, was studied. The volatile sulphur secondary substances emitted by *Allium* were tested in an olfactometer for their attraction from a distance on both sexes. Propylthiosulphinate proved to be highly attractive. Several C_6 general green leaf volatiles and epicuticular waxes were also tested. Sulphur chemicals perceived by olfaction have little effect on oviposition. Contact chemóreception is indispensable. Methanolic extracts of leek act in the same way as leek leaves. Of the sulphur substances and their precursors perceived on contact, only the slightly volatile thiosulphonate significantly stimulates oviposition. An extract of epicuticular waxes characteristic of *Allium* acted in the same way. The stimulation of oviposition seems due to several synergetic factors, among which the secondary volatiles specific to *Allium* play only a slight role. Rather, these volatiles, especially the more labile thiosulphinates, influence the leek moth female in the host plant orientation phase.

Key-words: Acrolepiopsis assectella, leek, attraction, oviposition, plant odours, plant waxes

Leek moth larvae, *Acrolepiopsis (Acrolepia) assectella* Zell. (Lepidoptera: Hyponomeutoïdea) develop as miners only in the parenchyma of *Allium*. Moving about with difficulty, they cannot search far for their food after hatching so the female deposits her eggs directly on the host plant. Therefore host selection is primarily the responsibility of the female. We endeavoured to find the substances which permit this specialization of oviposition behaviour. Oviposition is characterized by a primary phase of attraction from a distance followed by a recognition phase with or without oviposition.

Several substances synthesized by *Allium* are well known such as its epicuticular waxes (Cassagne & Lessire, 1974) and its sulphur compounds (Schreyen et al., 1976). In particular, secondary sulphur volatiles are emitted into the atmosphere when the leaves are broken (Virtanen, 1965). An enzymatic reaction, beginning from alkylcysteine sulphoxide precursors produces labile alkylthiosulphinates (Ti) which, depending on environmental conditions, disproportionate more or less rapidly into stable alkylthiosulphonates (To) and disulphides (Ds) (Auger & Thibout, 1979). There are four alkyl moieties including propyl (Pr), methyl (Me) and allyl (Al).
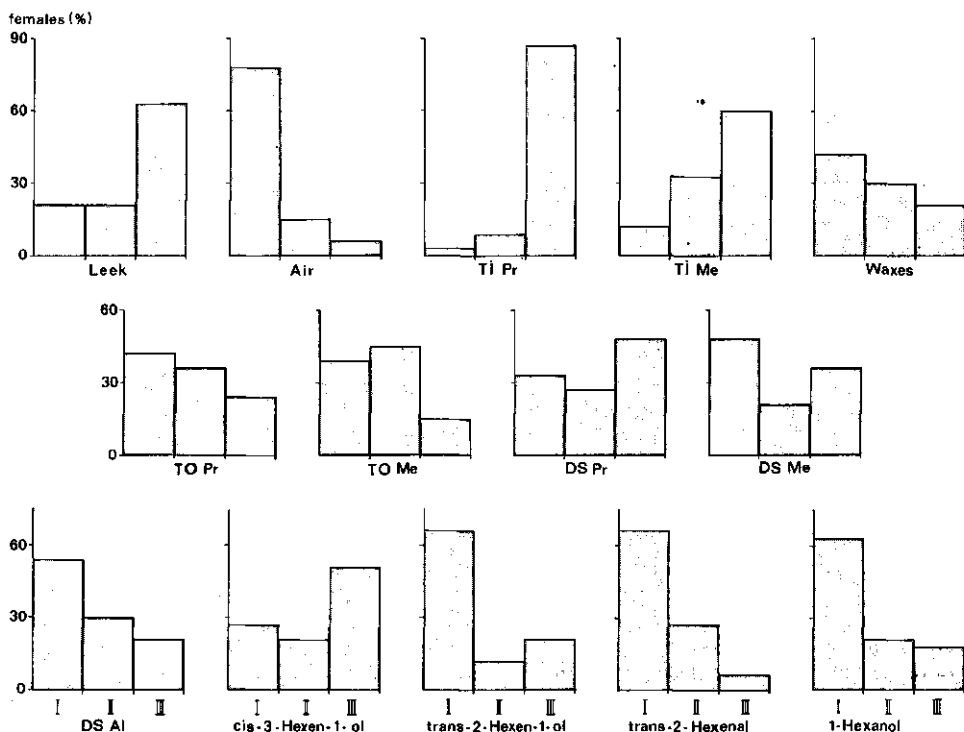
Fig. 1. Distribution of 30 virgin females placed separately in an air current coming from compartment III, with or without 'odour', 1 min. after being placed in compartment I. Ti Pr: propylthiosulphinate; Ti Me: methylthiosulphinate; To Pr: propylthiosulphonate; To Me: methylthiosulphonate; Ds Pr: propyldisulphide; Ds Me: methyldisulphide; Ds Al: allyldisulphide.

Attraction phase

With an olfactometer, we tested certain secondary substances of the leek *Allium porrum* L. (Liliaceae) for their attraction properties: propyl-and methylthiosulphinates and thiosulphonates (Ti Pr, Ti Me, To Pr and To Me); propyl, methyl and allyldisulphides (Ds Pr, Ds Me and Ds Al); four C_6 general green leaf volatiles and an odorous epicuticular wax extract. After 1 min, the distribution of the virgin females in a tube separated into three compartments revealed the attracting powers of each product (Figure 1; Lecomte & Thibout, 1981). A statistical analysis of the overall distribution distinguished three major categories: first the attractants Ti Me, cis-3-hexen-1-ol, leek, and Ti Pr; secondly, substances with little attracting effect but different from pure air, such as the To compounds, Ds Pr, Ds Me, and the waxes (Wx); and finally, chemicals with no effect such as Ds Al, trans-2-hexen-1-ol, trans-2-hexenal and 1-hexanol, all four comparable to pure air. Practically the same situation was found with the males except that the Wx were comparable to pure air.

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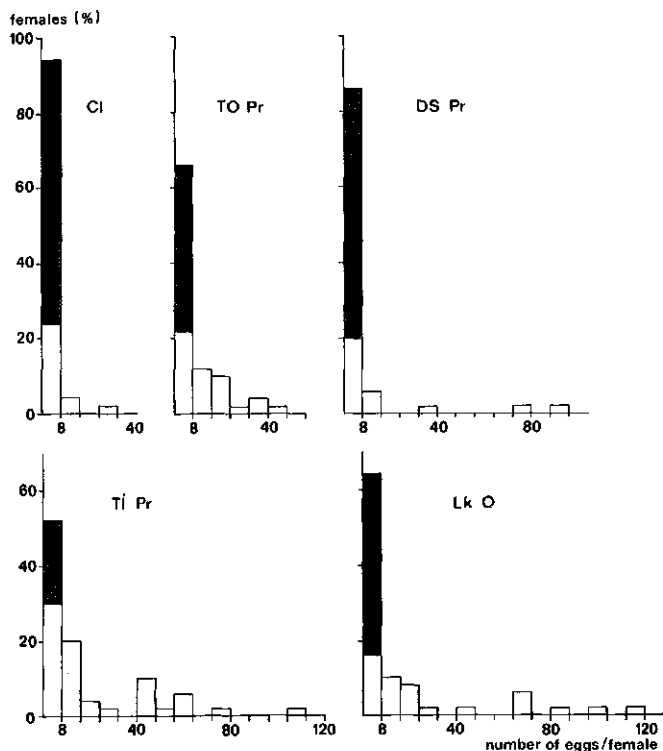
Fig. 2. Frequency distribution of the number of eggs laid per female in the presence of volatile sulphur secondary substances in the degree of oxidation of the sulphur atom. Role of olfaction. Dark bars indicate the percentage of non-ovipositing females. Cl: control (pure air); Ds Pr: propyldisulphide; Ti Pr: propylthiosulphinate; To Pr: propylthiosulphonate; Lk O: leek odour.

The most attractive moieties of sulphur substances in diminishing order are the Pr, the Me, then the Al. The preponderance of the labile Ti over the stable Ds and of the Pr over the Me is confirmed by electroantennographic studies (Pouzat, pers. comm.).

Oviposition and olfaction

As several of the above volatiles were attractive, we examined their effects on oviposition. Some were tested on isolated mated females (Figure 2; Auger & Thibout, 1979). Because the distribution of females was not normal, the results were compared by the Mann Whitney U and X^2 tests. Compared to oviposition in experiments, To and especially Ti, as well as the odour of leek slightly stimulated oviposition without stimulating chemicals and the Pr moieties were distinctly more active than Me (Figure 3). Ti Al had no effect since it disappears rapidly because of molecular instability. These results have been verified by electroantennography.

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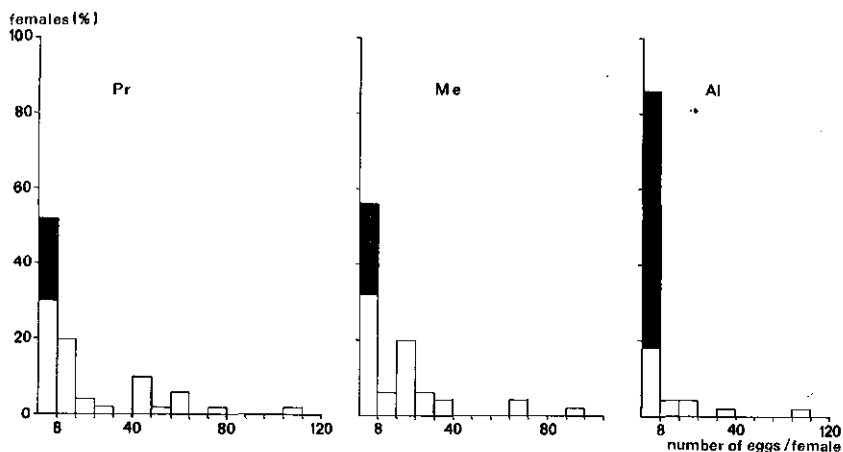
Fig. 3. Frequency distribution in the presence of different thiosulphinates. Comparison of alkyl moieties perceived by olfaction. Dark bars indicate the percentage of non-ovipositing females. Al: allyl; Me: methyl; Pr: propyl.

However, even with the most active of the volatile products, Ti Pr, or with leek odour, many females laid few or no eggs. Olfactory perception thus was insufficient and contact chemoreception as well as the hygrometric condition of the oviposition substrate seem very important (Thibout, 1974).

Oviposition and contact chemoreception

Perception of certain chemicals seems to be decisive as proved by experiments with isolated mated females in the presence of wet filter paper brushed with different extracts. Methanolic solutions of crushed leek presented in this way provoked egglaying as abundant as with a piece of leek (Figure 4). Solutions obtained by heating seemed more stimulatory than cold solutions. Two strains were equally sensitive. The results show excellent reproducibility and there was no aging of the extracts. Some contact chemoreception was implied since 86% of the eggs were deposited on the filter paper as opposed to 61% with the controls. This also shows that physical factors linked to leaf structures were of little importance.

Oviposition and precursors

Among the precursors of volatile substances, propylcysteine sulphoxide (PCSO) is in particular, one of the chemicals specific to the leek present in methanolic solutions. PCSO was synthesized and tested on mated females. Whether in pure form or in aqueous solution on paper or on iris leaf, it did not stimulate oviposition in *A. assectella* (Auger & Thibout, 1981a). These results were confirmed by studying oviposition of mated females placed with a piece of Liliaceae leaf dipped in an aqueous solution of PCSO (Table 1). Other than with leek, the presence of PCSO re-

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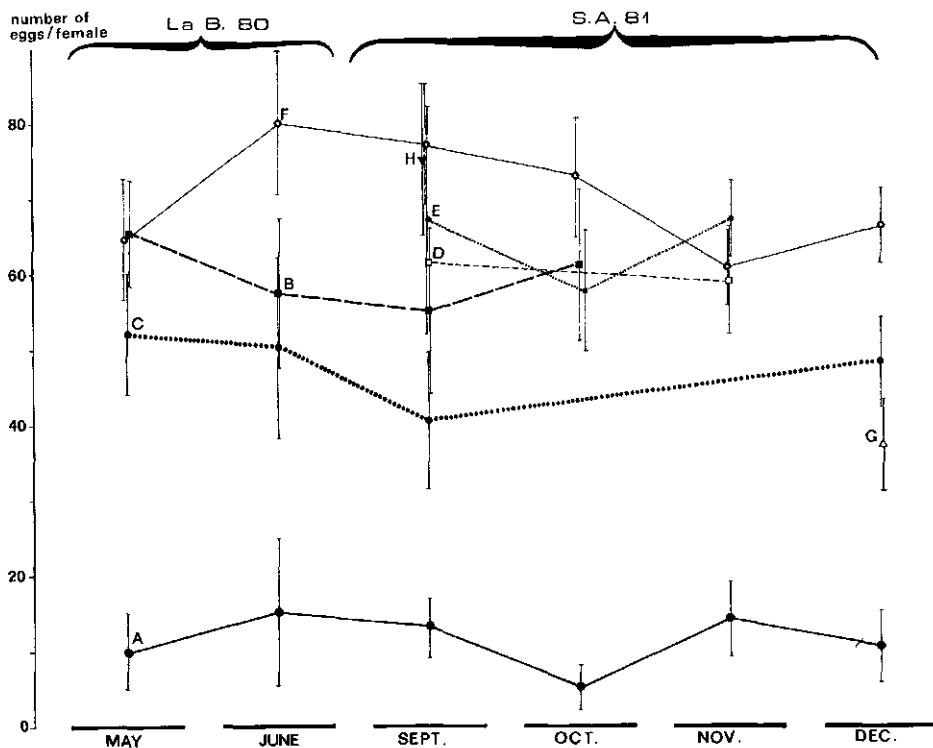
Fig. 4. Oviposition by isolated females (mean ± standard error) of two strains during the year, in the presence of different methanolic extracts of leek. A: control (water); B: hot methanolic extract 1; C: cold methanolic extract 1; D: hot methanolic extract 2; E: cold methanolic extract 2; F: leek leaf; G: extract with CH_2Cl_2; H: B retaken with H_2O; LA B.80: Strain La Bourdaisière 1980; S.A. 81: Strain St-Avertin 1981.

Table 1. Number of eggs per female and the percentage deposited on the substratum (paper or Liliaceae leaf) with or without propylcysteine sulphoxide (PCSO).

	Paper	Leek	Jacinth	Iris
H_2O				
Number of ♀	27	24	22	21
Eggs per ♀	34.9	109.6	51.2	57.4
Percentage eggs	77	98	85	64
H_2O + PCSO				
Number of ♀	33	16	33	17
Eggs per ♀	24.0	119.9	34.1	12.4
Percentage eggs	42	94	67	17

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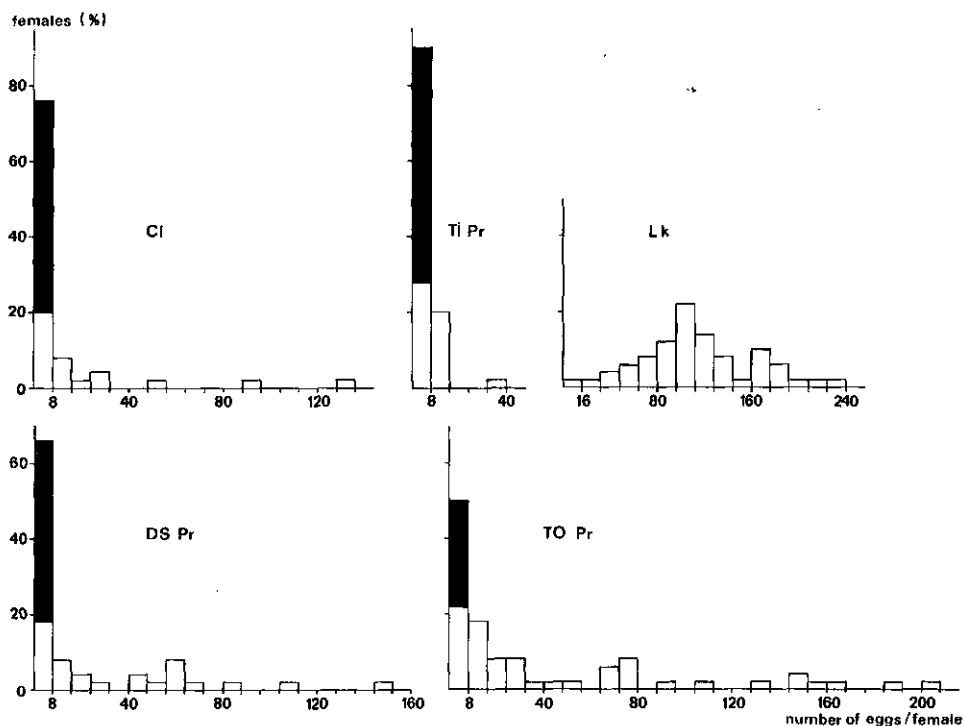
Fig. 5. Frequency distribution of the number of eggs laid per female in the presence of secondary volatiles of leek containing propyl moieties, percieved by contact. Dark bars indicate the percentage of non-ovipositing females. Cl = control (wet paper); Ds Pr = disulphide solution; Ti Pr = thiosulphinate solution; To Pr = thiosulphonate solution; Lk = leek leaf.

duced egglaying, particularly the number of eggs deposited on the substratum. Thus, this isolated precursor inhibits oviposition. We therefore went on to seek other host-specific contact chemostimulants.

Oviposition and volatile substances perceived by contact

The sulphur secondary volatiles were tested in water solution for their effect on oviposition (Figure 5). The distribution of mated females in the presence of Ds Pr and Ti Pr was not different from control females whereas the distribution with To Pr was significantly different. To Pr, the least volatile of the substances studied, thus seems to stimulate oviposition following perception by contact. However, this product does not act alone since many more eggs are produced in the presence of leek.

Oviposition and waxes

Because the female leek moth deposits her eggs on the surface of the vegetable, we collected its epicuticular waxes by rinsing leek leaves with methylene chloride.

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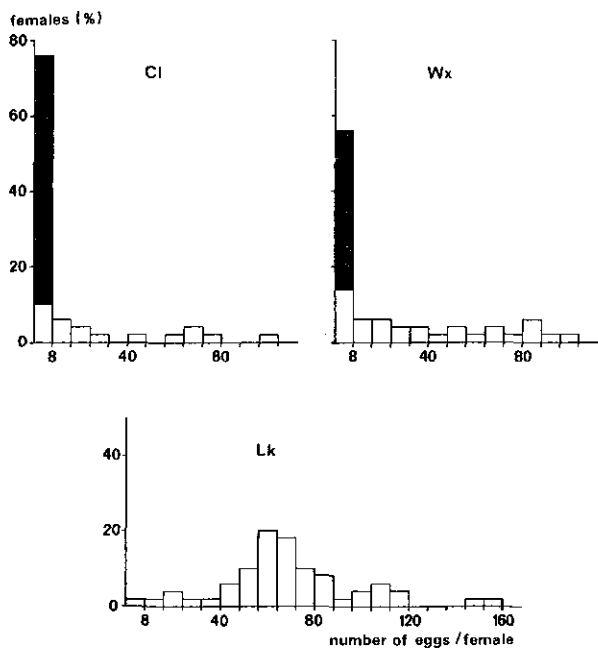
Fig. 6. Frequency distribution of the number of eggs laid per female in the presence of an extract of epicuticular waxes (Wx). See for other legend Figure 5.

The extract was then applied to wet filter paper and exposed to isolated mated females (Figure 6). Contact with leek provoked a sizeable egg deposition. The distribution of mated females producing eggs in the presence of waxes was different from the distribution of controls although the number of eggs was low in both cases. Of the eggs 72% was deposited on the wet paper impregnated with the waxes whereas, with the controls, the percentage dropped to 47%. The experiments with the different extracts show that oviposition is to some extent, stimulated by contact with leek epicuticular waxes. Egglaying remains highly variable as it can reach 60 eggs per female (equal to the oviposition obtained with leek) or be as low as 5 eggs per female (similar to the result obtained with controls). This might be caused by variability in female responses as the same extract used in several experiments can induce very different proportions of oviposition. Thus, waxes and thiosulphonates, are not the sole factor causing oviposition.

Conclusion

Females and males of *A. assectella* are attracted by the odour of leek and respond to certain volatiles constituting this odour. Cis-3-hexen-1-ol and the Ti compounds, especially Ti Pr, prove to be the most attractive. This confirms the hypothesis that the unstable Ti compounds are better spatio-temporal chemical messengers than other stable secondary substances of the leek (Auger & Thibout, 1979). Their un-

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stability avoids an accumulation in the atmosphere, detrimental to the precision of the information provided by them. Moreover, although rarely observable by GC, Ti Pr has been identified in the odour of leek by MS (Auger & Thibout, 1981b). The activity of the secondary sulphur substances is probably not linked to the nature of its carbon chain, although molecules with the propyl moieties are the most efficient (Lecomte & Thibout, 1981). On the other hand, the degree of oxidation of the sulphur atoms seems very important. The Ti and the To products have greater effects on attraction and oviposition than the Ds. Certain species of specialist phytophagous Diptera deposit their eggs in the soil next to their host plant and are sensitive to secondary volatile substances (Sömme & Rygg, 1972; Finch, 1976; Städler, 1978; Vernon et al., 1978). On the other hand, as *A. assectella* deposits its eggs directly on the host plant, oviposition is hardly stimulated by the volatile secondary substances of leek. Chemical perception through contact with the tarsus (Dakkouni & Thibout, 1981) is indispensable for the correct progression of oviposition behaviour in the leek moth. Contrary to results obtained in *Pieris brassicae* (Ma & Schoonhoven, 1973) with sinigrin, a precursor of volatile secondary substances of Cruciferae, oviposition of *A. assectella* is inhibited by the precursor of volatile secondary substances of leek, PSCO. Among the sulphur substances specific to *Allium*, only To Pr seems to stimulate oviposition. The recognition of the host plant by *A. assectella* females, indispensable to the survival of their progeny is then due to contact chemoreception of several substances acting in synergy. Among these factors, slightly volatile chemical substances such as To Pr and the waxes play an important role. The To, produced by a specific enzymatic reaction, are rare other than in *Allium* and the fatty acid and alkane composition of the epicuticular waxes is peculiar to each vegetable species. These substances therefore seem to facilitate host selection.

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Synergism of visual and chemical stimuli in the oviposition behaviour of Delia antiqua

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Abstract

Stimuli emanating from onion stems are important in releasing *Delia antiqua* oviposition behaviour. The role of visual/structural stimuli from stems was assessed using glass tubes containing coloured paper. These tubes competed favourably with onion stems when both were presented with chemical stimuli. When visual (yellow paper), structural (glass tubes), and chemical (chopped onion) stimuli were presented separately and in various combinations, treatments combining all three stimuli elicited about 80% of the total oviposition. Inasmuch as treatments containing either chemical stimuli or visual/structural stimuli alone received only about 3% of the eggs, combinations of visual, structural, and chemical stimuli have a synergistic effect on oviposition. A synergistic effect was also observed when chemical and structural stimuli were combined, indicating that the onion stem provides not only requisite colour stimuli, but also a stage for the performance of post-alighting pre-oviposition behaviours.

Opinions on the amount and diversity of information used by insects finding and accepting host plants are shifting. The view that host range is determined by the presence of a few 'key' chemical stimulants (Dethier, 1954) or absence of particular deterrents (e.g. Jermy, 1958; Thorsteinson, 1960) is yielding to the view that insects respond to a summation of inputs from receptors sensitive to a range of plant constituents (Dethier, 1971). This summated signal ('Gestalt') is believed to arise via across-fiber patterning processes. Furthermore, the finding that larval Lepidoptera specialists had greater receptor sensitivity to plant saps than did a representative non-herbivore (the blowfly, *Phormia regina*), led Dethier (1980) to suggest ways that insect herbivores receiving larger amounts of chemically derived information from potential hosts might realize an adaptive advantage. Implicit in these arguments is the understanding that sensitivity sets the stage for evolution of adaptive behavioural responses to complex chemical blends which vary subtly but ecologically significantly.

This generous view of insect perceptual capabilities might also be applied across sensory modalities. Although it makes sense theoretically that insects would use in-

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formation from any available sensory channel when examining and accepting hosts, the potential interplay across sensory modalities is often overlooked.

The role of chemicals in the onion *Allium cepa* L./onion fly *Delia antiqua* (Meigen) (Diptera: Anthomyiidae) relationship has been explored in detail. Matsumoto & Thorsteinson (1968) demonstrated that *n*-dipropyl disulfide and propanethiol (major volatile constituents of onion) applied to moist sand released oviposition by *D. antiqua*. Since then, a number of *Allium* alkyl sulfides and thiols as well as their analogues have been bioassayed. Arising therefrom has been the hypothesis that *D. antiqua* oviposition stimulants must contain a sulfur atom possessing 2 unshared electron pairs and bonded to a saturated hydrocarbon chain 3-5 carbons long (Ishikawa et al., 1978; Vernon et al., 1978). In addition to the stimulants from onion, several microbially produced synergists of the alkyl sulfides have been reported (Ikeshoji et al., 1980), including ethyl acetate and tetramethyl pyrazine.

Although the role of chemicals in this 'classic' insect-plant relationship has received much attention, the importance of other cues and sensory modalities has gone relatively unexplored. We have re-examined this relationship to determine whether visual/structural stimuli significantly modulate oviposition responses to host chemicals.

Materials and methods

Parental stock for the culture of *D. antiqua* was obtained from Dr. F. McEwen, University of Guelph, Ontario, Canada. Flies used here, 8-10 generations removed from the field, were housed in 60 × 60 × 80 cm screened cages provisioned with water, honey, and the diet of Ticheler (1971); larvae were reared on onions.

Treatments to be bioassayed for stimulation of *D. antiqua* oviposition were placed in cages containing several hundred reproductively mature flies. During experiments (July through October, 1981), cages were placed either outdoors or in a glasshouse, depending on weather. The design of all experiments was randomized complete block with one block per cage per 24 hours sampling time. Rather than completing all blocks of a given experiment and then moving to another experiment, one block of every experiment was completed in random order and the cycle repeated. This precaution was taken to avoid training effects possible with flies (Prokopy et al., p.123) repeatedly exposed to the same treatments. Eggs were collected from the sand by flotation, counted, and recorded as percent of the total laid in the block. Resulting data did not fulfill the assumptions of normality either with or without transformations. Therefore, results were analyzed using the nonparametric equivalent of the F-test, Kruskal-Wallis one way analysis of variance by ranks (Siegel, 1956).

In an experiment comparing the relative importance of above- vs below-ground onion plant stimuli, small sprouted onions (cultivar Abundance) with 2-3-cm-diam bulbs and 5-7-cm-long stems were cut into 2 parts, stem and bulb. The bulb was submerged in a cup, 8 cm in diam and 5 cm deep, filled with 200 ml of washed silica sand containing 50 ml water. The stem was placed in a second cup so that it rose vertically from the sand and presented a visual/structural stimulus similar to young

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onions in the field. A third cup, containing only sand and water, served as a control.

The importance of the interaction between chemical and visual/structural cues from onion stems was investigated by fashioning a surrogate stem and comparing oviposition around it with that around an onion stem of similar size and shape. Eight-cm sections, cut from 5 to 8-mm-diam onion stems, served as authentic above-ground onion stimuli. Surrogate stems consisted of 8-mm OD Pyrex TM glass tubes cut in 8-cm sections and having a heat-sealed tapered end. The open end was sealed with a cork and submerged in the sand during experiments. As needed, a 7 × 0.5 cm strip of yellow silkscreened paper (Geller Artists, New York, NY) coloured on both sides was inserted into the glass tube to provide colour or brightness stimuli. Two stems, either onion or surrogate, were inserted so they stood 6.5 cm tall and 5 cm apart in the sand contained in each 7.5 × 7.5 × 2.5 cm oviposition dish bearing 5 ml of submerged chopped onion as the chemical stimulus. Sand moisture was stabilized via a filter paper wick contacting a water reservoir (Harris & Miller, 1982).

Results and discussion

Initial experiments comparing oviposition on stems vs bulbs included an intact onion plant; however, as 80-90% of the eggs were laid on this treatment, we removed it subsequently to force females to oviposit either on onion stems or submerged bulbs. When given only these treatments and a control, flies laid 18 times more eggs around the stems (Table 1), demonstrating the importance of above-ground cues. While the results of this experiment did not indicate whether the stem's chemical or visual/structural stimuli played the major role in eliciting oviposition, work by Pierce et al. (1978) suggests that stem chemical stimuli were not solely responsible; pentane extracts of onion bulbs elicited 16 times more oviposition than did extracts of either onion stems or leaves.

There were no significant differences between the number of eggs laid on onion stems and surrogate stems as long as they were presented with chopped onion in the sand (Table 2); both received about 20 times more eggs than the control with chopped onion but no stems. In the absence of subterranean chopped onion, onion stems

Table 1. Relative importance of above-ground vs below-ground onion stimuli for oviposition by the onion fly.

Treatment	Percentage of total eggs laid[1]
Bulb submerged in sand	5.3b
Stem standing in sand	94.7a
Sand control	0.0c

1. Mean of three replicates; total number of eggs is 786; means followed by differing letters are significantly different at $P \leqslant 0.05$.

Table 2. Relative effectiveness of onion stems vs surrogate stems both with and without subterranean chopped onion in stimulating oviposition by the onion fly.

Above ground stimulus	Percentage of total eggs laid [1]	
	below-ground stimulus	
	sand	onion
Onion stem	16.9b	43.3a
Surrogate stem	1.0c	36.7a
No stem	0.0d	2.1c

1. Mean of five replicates; total number of eggs is 6185; means followed by differing letters are significantly different at $P \leqslant 0.05$.

(which provided some above-ground chemical stimuli) received significantly more eggs than surrogates, but significantly less than stems with chopped onion in the sand (Table 2). Thus, *D. antiqua* oviposition is apparently influenced by visual and chemical cues from onion stems as well as by onion chemicals in the soil.

We tested chemical and visual/structural stimuli both separately and in various combinations. Six different treatments were presented in one experiment: sand alone, sand with chopped onion (chemical alone), clear stems in sand (structural alone), clear stems in sand with chopped onion (structural and chemical), yellow stems in sand (structural and visual), and yellow stems in sand with chopped onion (visual, structural and chemical). Combining the 3 stimuli had a pronounced synergistic, rather than additive, effect on oviposition (Table 3). Few eggs were laid on treatments containing only chemical, structural, or visual/structural stimuli singly; however, the addition of a clear glass tube to a dish containing only moist sand and chopped onion caused a synergistic increase in the number of eggs laid (from 0.4 to

Table 3. Relative importance of chemical, visual and structural stimuli for oviposition by the onion fly.

Visual/structural stimulus	Percentage of total eggs laid[1]	
	chemical stimulus	
	nothing	chopped onion
No stems	0.0c	2.8c
Clear glass stems	0.4c	15.8b
Yellow glass stems	2.8c	78.1a

1. Mean of four replicates; total number of eggs is 2832; means followed by differing letters are significantly different at $P \leqslant 0.05$.

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15.8% of the total). This suggested that the onion stem elicits oviposition not only because it reflects light of stimulatory wavelengths, but also because it presents a structure allowing performance of requisite pre-oviposition behaviours. Preliminary observations indicate that *D. antiqua* females almost invariably spend several minutes walking up and down onion and surrogate stems before probing the sand with their ovipositors and eventually laying eggs.

The mechanisms behind the synergistic effects of combined chemical and visual/structural cues in stimulating *D. antiqua* oviposition are not known. These stimuli might operate either sequentially or simultaneously. *D. antiqua* might, for instance, respond anemotactically, klino-, or orthokinetically to olfactory stimuli, and upon reaching the oviposition site, encounter visual/structural stimuli which elicit oviposition in the presence of chemo-tactile stimuli. Alternatively, the presence of olfactory stimuli might cause the fly to respond more strongly to visual stimuli. Vaidya (1969) found that the lemon butterfly, *Papilio demoleus*, did not respond to isolated citrus odours or colours; however, placing an odour source near the coloured papers elicited drumming, an important phase in examining the host before oviposition by this species.

In summary, the behavioural repertoire of the gravid onion fly is richer than once envisioned. Although chemicals are one of the fundamentally important cues stimulating egg deposition, visual/structural cues also feature in this insect plant interaction. The ecological significance of *D. antiqua*'s responsiveness to multichannel cues remains to be elucidated. Working hypotheses could reasonably focus on enhanced finding and acceptance of the better hosts available, as well as better allocation and placement of eggs on each host. The increased behavioural plasticity, possible when responsive to multiple cues, might be especially important to polyvoltine insects like *D. antiqua* whose host plants vary markedly over a growing season.

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Variation in host acceptance pattern in apple maggot flies

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Abstract

Lab and field assays of propensity of apple maggot flies, *Rhagoletis pomonella*, to attempt oviposition in different types of host fruit revealed a highly variable acceptance pattern. The assays suggest (a) genetically-based differences in acceptance pattern between flies whose larvae were of apple origin vs. *Crataegus* origin, (b) alteration of acceptance pattern through selection over many generations, (c) lack of substantive influence of induction during the larval stage on acceptance pattern, and (d) considerable influence of induction during the adult stage (prior ovipositional experience) on acceptance pattern.

Key-words: Rhagoletis pomonella, oviposition, host race formation, induced food preferences

The host preference pattern of herbivorous insects may be fixed or variable (Fox & Morrow, 1981). If fixed, then all individuals of all populations should manifest the same rank order of choice among potential hosts, unmodified by local populational or environmental conditions. To date, there are few examples of this sort (Fox & Morrow, 1981).

A variable preference pattern appears to be the more common one. The variation may be partitioned into genetic and non-genetic components. If principally under genetic control, then one would expect the preference pattern to remain consistent within an individual or population, as seems to be the case in several species (Singer, 1971; Hsiao, 1978; Mitter et al., 1979; Wiklund, 1981; Tabashnik et al., 1981). If principally under non-genetic control, then an array of local factors may modify the preference pattern. These should include:
— insect physiological state (Singer, 1981; Wiklund, 1981)
— phenology, size, density, and quality of hosts (Singer, 1971; Wolfson, 1980; Rausher et al., 1981; Futuyma & Saks, 1981; Risch, 1981)
— competition effects among insects (Wasserman, 1981)
— effects of predators or parasitoids (Smiley, 1978)
— previous experience of the herbivore as an immature (Jermy et al., 1968; Wiklund, 1973; Cassidy, 1978; Dethier, 1980) or adult (Phillips, 1977; Rausher, 1978; Traynier, 1979).

In some cases, variation in host selection pattern may result from substantial intra- or interpopulational genetic differences which are selectable through varying local conditions (Gould, 1979; Wasserman & Futuyma, 1981).

The apple maggot fly *Rhagoletis pomonella*, is an indigenous North American insect whose principal native host is hawthorn (*Crataegus*). During the past 120 years, the host range of this insect has broadened to include fruit of the following introduced plants: apple, domestic plum, sour cherry, apricot, pear, and *Rosa rugosa* (Prokopy & Berlocher, 1980). Considerable is known about the composition of chemical and physical host plant stimuli involved in oviposition site detection by apple maggot fly (Prokopy, 1977), and Bush (1974) has provided an elegant hypothesis of the process of apple maggot fly host range expansion and possible host race formation. Some research has been conducted on degree of oviposition by this fly into known and potential host fruit under lab (Hall, 1938; Pickett & Neary, 1940) and field (Reissig & Smith, 1978) conditions, the results of which suggest that the flies whose larvae originated from either apple or *Crataegus* are capable of ovipositing into both of these and several additional species of fruit. Few of these experiments, however, involved testing responses of individual apple maggot flies of known ovipositional history.

Here, we report on the ovipositional response of naive and experienced individually-assayed apple maggot flies of different populations to *Crataegus*, apple, and *R. rugosa* fruit under lab and field conditions.

Materials and methods

All *R. pomonella* assayed in lab tests originated from puparia formed by larvae which infested apples collected from unsprayed trees in Amherst or Conway, Mass., *Crataegus mollis* collected from unsprayed trees in Amherst, or apples maintained in cages under lab conditions, wherein apple maggot flies had been confined (without exposure to any other fruit type) for about 50 generations. Both sexes were maintained together in cages supplied with food (sucrose and yeast hydrolysate) and water at about 25°C, 50% RH, and 18-hour photoperiod. Females were assayed at 2-3 weeks of age, when mature and presumably mated. At time of assay initiation, all flies were naive (without previous exposure to any fruit). Each was selected at random from a maintenance cage and gently transferred to a test cage devoid of any except assay fruit. Each was offered (allowed to walk onto) a single assay fruit and permitted to remain there until it either accepted (attempted oviposition) or rejected (left without attempting oviposition) the fruit, or until 10 min had elapsed without acceptance or rejection (these were excluded from data analysis). In nearly all cases, acceptance or rejection occurred within the first 5 min. In one set of experiments (Table 3), we experimentally trained naive apple maggot flies whose larvae were of apple origin (collected in nature). Training consisted of 4 successive ovipostions (2 min apart) on fruit of the same type. Two min later, the fly was offered a 5th fruit of same or different type.

In the field tests, all apple maggot flies assayed had just (within 5 min) finished ovipositing on growing 'Red Delicious' or *C. mollis* in the same trees from which larval collections were made the previous year. While still on or close to the fruit in

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Table 1. *Rhagoletis pomonella* acceptance of 4 fruit types offered under lab (Exp. 1) and field (Exp. 2) conditions (1 fruit type/fly; 50 flies/treatment).

Exp.	Flies	Females accepting fruit (%)[1]			
		C.mollis	*R.rugosa*	'McIntosh'	'Red Del.'
1	Naive, apple origin	90a	86a	48bc	36cd
	Naive, *C.mollis* origin	90a	60b	14e	26de
2	Experienced on Red.Del.	0c	8bc	12b	62a
	Experienced on *C.mollis*	60a	20b	0c	16b

1. Values followed by the same letter are not significantly different.

which it had just oviposited, a fly was offered, under the same protocol as in the lab tests, a single assay fruit for acceptance or rejection.

Assay fruit consisted of uninfested 'Red Delicious' and 'McIntosh' apples, and fruit of *C. mollis* and *R. rugosa*. Each was picked fresh and stored at 3°C until needed. For assay, each was placed on a dissecting needle and presented to a fly. So long as the experimenter moved slowly, the insect's behaviour did not appear to be adversely affected.

Data were statistically analyzed using a G-test at the 0.05 level.

Results

In lab assays, naive apple maggot flies whose larvae were of apple origin accepted *C. mollis* and *R. rugosa* about equally (90 and 86%) and accepted 'McIntosh' and 'Red Delicious' to a significantly lesser extent (Table 1, Exp. 1). Naive, lab-assayed *R. pomonella* whose larvae were of *C. mollis* origin likewise exhibited 90% accep-

Table 2. *Rhagoletis pomonella* acceptance of *C. mollis* or 'Red Delicious' in lab assays (each fly offered each fruit type, 10 min apart). + = acceptance, − = rejection.

Larval origin of flies	Numbers assayed	Females in each category (%)[1]			
		C.mollis[2] +	+	−	−
		'Red.Del.'[3] −	+	+	−
Amherst *C.mollis*	100	83a	9ef	0g	8ef
Amherst apple	121	64b	24c	2fg	10ef
Conway apple	57	60b	21cd	2fg	18cde
Lab-cult on apple	114	24c	54b	9ef	13de

1. See footnote of Table 1.
2. Half of flies of each population offered *C. mollis* first, half offered 'Red. Del.' first.
3. 'Red. Del.' were from a different batch than in Table 1.

Table 3. *Rhagoletis pomonella* acceptance of a 5th fruit
after oviposition in each of first 4 fruit offered.

Exp.	First 4 fruit	Fifth fruit	Number of flies	Acceptance of 5th fruit (%)[1]
1	Apple	C.mollis	29	52a
	C.mollis	C.mollis	47	91b
2	C.mollis	Apple	49	20a
	Apple	Apple	26	92b

1. See footnote of Table 1.

tance of *C. mollis*, with significantly lesser acceptance of the other 3 fruit types
(Table 1, Exp. 1). Importantly, *R. rugosa* and 'McIntosh' were significantly more
acceptable to apple-origin flies than to flies of *C. mollis* origin.

In striking contrast, females which had just finished ovipositing on 'Red Deli-
cious' in nature accepted 'Red Delicious' to a significantly greater degree than any
of the other 3 fruit types (Table 1, Exp. 2). Analogously, females which had just
finished ovipositing on *C. mollis* in nature accepted *C. mollis* to a significantly great-
er degree than any of the other 3 fruit types (Table 1, Exp. 2).

In lab assays wherein each naive apple maggot fly was offered a 'Red Delicious'
and *C. mollis* fruit 10 min apart, a significantly greater percentage of each of the 4
populations tested (*C. mollis* from Amherst; apples from Amherst; apples from
Conway; lab-cultured on apples for about 50 generations) accepted *C. mollis* and
rejected 'Red Delicious' than accepted 'Red Delicious' and rejected 'C. mollis (Tab-
le 2). However, the proportion accepting *C. mollis* and rejecting 'Red Delicious' was
significantly greater among *C. mollis* origin flies than among Amherst or Conway
apple origin flies, wherein in turn it was significantly greater than among the lab-
cultered apple origin flies (Table 2).

Apple maggot flies trained on 4 'Red Delicious' rejected *C. mollis* as a 5th fruit to
a significantly greater degree than females trained on 4 *C. mollis* (Table 3, Exp. 1).
Similarly, flies trained on 4 *C. mollis* rejected 'Red Delicious' as a 5th fruit to a
significantly greater degree than flies trained on 4 'Red Delicious' (Table 3, Exp. 2).

Discussion

The combined results reveal a variable host acceptance pattern in ovipositing ap-
ple maggot flies and indicate the variation to stem from genetic as well as non-ge-
netic factors. Irrespective of wild population origin (apple or *C. mollis*), naive flies
demonstrated a significantly greater propensity to accept *C. mollis* than 'Red Deli-
cious'. However, naive *R. pomonella* whose larvae were of apple origin exhibited a
greater propensity to accept other host fruit types, such as *R. rugosa*, than did *R.
pomonella* whose larvae were of *C. mollis* origin. Together, these findings suggest
that induction during the larval stage had no substantive influence on this pattern of
host acceptance (Hopkins' Host Selection Principle) and that genetic differences be-

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tween flies of *C. mollis* and apple origin were partly (or mostly) responsible for this pattern. Assay results of naive *R. pomonella* whose progenitors were lab-cultured on apples for about 50 generations suggest that genetic differences among or within apple maggot fly populations are somewhat selectable. Table 3 results and additional fly training experiments reported in Prokopy et al. (1982) conclusively show that propensity of apple maggot flies to accept host fruit of a given type can be induced through prior ovipositional experience. Such induction, together with possible but yet undemonstrated effects of host tree experience prior to sexual maturity, may largely explain the total rejection of *C. mollis* by females which had just finished ovipositing on 'Red Delicious' in nature (Table 1, Exp. 2).

Our findings lend support to the hypothesis of Bush (1974) that genetically based differences in host preference pattern exist among different populations of apple maggot flies. To what extent such differences may play a role in the hypothesized host race formation process in this insect Bush (1974) is uncertain. Whatever, induction of host preference through prior adult experience may modify the expression of genetically based propensities in some instances.

The experimental approach taken here may be of value to investigators designing management practices for apple maggot flies in commercial orchards. For example, within the past 4 years, these flies were introduced for the first time into Oregon (AliNiazee & Penrose, 1982), where they presently infest apples and *Crataegus*. A pest manager in Oregon could benefit from knowing whether Oregon apple maggot flies have the same genetically-based potential of infesting *Rosa* and pear as those insects in Massachusetts. The Oregon manager could also benefit from knowing how susceptible the host preference pattern of Oregon flies were to selection, and thus how rapidly, if at all, the preference pattern might change over time. Finally, the Oregon manager could benefit from knowing how strongly induction through prior adult experience might influence apple maggot fly foraging behaviour (Roitberg et al., 1982), and the distance over which a fly might move in search of a patchily distributed host type on which it had been induced.

Acknowledgment

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The role of microorganisms colonising radish seedlings in the oviposition behaviour of cabbage root fly, Delia radicum

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Abstract

Experiments were carried out to investigate whether cabbage root fly (*Delia radicum*) egg-laying behaviour was influenced by microbial activity in or around its host plants. Various antimicrobial agents were used alone or in combination to kill microorganisms on radish seeds. Some treatments modified the composition of the microflora, while others were effective in reducing microorganisms to low levels. Radish seedlings from treated and untreated seeds lots were raised in sterile sand in glass containers and presented to flies in laboratory test cages. Flies laid up to three or four times as many eggs around plants grown from untreated seed as compared to those carrying little or no microorganisms. Plants grown from treated seeds which were 're-inoculated' with microorganisms received as many eggs as untreated controls. The implications of these findings in relation to the ecology of cabbage root fly are discussed.

Key-words: Delia radicum, oviposition, microorganisms, seed treatments, radish, plant resistance

In recent years there has been an intensification of the search for alternative methods of cabbage root fly (*Delia radicum* L.) control to be used as supplements to insecticides. At Wellesbourne several different alternatives have been or are being studied, for example, protective barriers to egg-laying, sterilisation and host plant resistance. Basic to all these studies is a knowledge of insect-plant relationships and, in particular, host selection. Indeed, survival of the insect depends on the ability to locate and lay eggs around host plants. Like most plant-feeding insects cabbage root fly is believed to locate its cruciferous host by responding to the volatile chemicals released from the plant. These volatile chemicals which give the crop its characteristic odour, were suggested as early as 1925 by Glasgow to be attractants to the fly but it was not until much later that cabbage root fly activity was shown to be increased by these chemicals (Traynier, 1967). The attraction of cabbage root fly to its host crops has been studied by several authors, for example, Finch (1978), Hawkes & Coaker (1979) and Städler (1978). One aspect of host selection which has received little attention is the mechanism by which the volatile chemicals are made available to the flies. Microorganisms colonising the host plant have been shown to influence

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the egg-laying behaviour of the bean seed fly, *Delia platura* (Meigen) (Eckenrode et al., 1975) and the onion fly, *Delia antiqua* (Meigen) (Ellis et al., 1979). In this paper we report laboratory investigations of the role of microorganisms in the relationship between cabbage root fly and one of its host crops, radish (*Raphanus sativus* L.).

Materials and methods

Production of microbe-free plants   Several methods were investigated to eliminate microorganisms from seeds prior to germination and growth in a microbe-free environment. These included relatively complex methods involving combinations of bactericides, antibiotics and fungicides and the use of partial vacuum to enable penetration of the compounds into seeds (Barber, 1967; Ellis et al., 1979) and also a simple soaking technique with antibiotics and sodium hypochlorite (NaOCl).

All treatments and subsequent manipulations were carried out under microbe-free conditions on a sterile air bench. Seeds were treated in 50-seed lots contained in nylon gauze. Both treated and untreated seeds were then placed on moist filter paper in sterile Petri dishes (50 seeds/dish) and incubated for 2 days at 25°C to germinate before transfer to 250-millilitre pyrex glass beakers. Each beaker contained 65 g of washed dry sand moistened with 15 ml of water (with a temporary covering of aluminium foil to maintain sterility after autoclaving), and was sterilised by autoclaving at 121°C for 20 min. The germinating seedlings were transferred with sterile forceps to the surface of the moist sand in the beakers and covered with a thin layer of dry sterile sand. The beakers were then re-sealed with sterile transparent polypropylene film and incubated at 20°C with an 18-hour photoperiod provided by fluorescent tube illumination. Initial experiments were made with 40 to 50 seedlings per beaker but in later experiments either one or 10 seedlings were grown in each beaker.

Tests of microbial sterility were made by a dilution plate technique. Five seedlings from each beaker were macerated in 2 ml of nutrient broth in a sterile Griffith tube and then 0.1-ml aliquots of the extract and a number of serial 1:10 dilutions of the extract were plated onto nutrient agar. Estimates of microbial numbers were made from colony counts after 2 to 3 days incubation at 25°C.

Of the treatments examined, the following were used most extensively:
— The method of Ellis et al. (1979) whereby seeds were immersed in a mixture containing thiram of concentration 2 g/l and vancomycin at a 0.5 g/l and subjected to a partial vacuum (0.13 kPa) for 24 h. After this the seeds were rinsed twice in a solution of NaOCl containing available chlorine of concentration 9 g/l, followed by two rinses in sterile water.
— The method of Humaydan et al. (1980) whereby seeds were immersed for 1 to 2 h in solutions containing either streptomycin or vancomycin of concentration 0.5 g/l followed by a single rinse in sterile water and a soak for 30 min in NaOCl (available chlorine 5 g/l).
— Immersion for 1 to 2 h in a solution containing streptomycin and polymyxin B both at 0.5 g/l followed by a single rinse in sterile water and a soak for 30 min in NaOCl (available chlorine 5 g/l).
— Immersion for 1 h in NaOCl (available chlorine 25 g/l).

Using antibiotic-impregnated discs, limited tests were made of the antibiotic sensitivity of a range of bacterial isolates obtained from treated and untreated seedlings.

Cabbage root flies   The cabbage root flies used in all experiments were obtained from a continuous laboratory culture maintained at Wellesbourne (Finch & Coaker, 1969). In all tests 300-400 female and 100 male, 4- to 7-day-old flies were used, these flies being equally divided into six groups for experiments involving the series of small cages.

Test cages   Plants were exposed to the flies in a single large test cage or in six separate cages. The large test cage, 1.5 × 1.5 m and 1.35 m high, was fitted with Tygan mesh sides and glass doors and contained a 1.45-metre diameter turntable which rotated once every 4 min (Ellis & Hardman, 1975).

The six small test cages, 0.35 × 0.35 × 0.35 m, were constructed from a wooden ball-and-rod frame surrounded by terylene netting. The cages were arranged beneath 400-watt mercury fluorescent lamps in the controlled environment room and surrounded by white card screens (0.63 m high, 1.45 m diam.) to increase the uniformity of illumination. Each cage contained at least one replicate of each treatment in an experiment. All cages were housed in a controlled environment room maintained at $18 \pm 1°C$, $65 \pm 5\%$ R.H. which received a 16-hour photoperiod.

Experiments   A series of experiments were conducted to investigate cabbage root fly egg-laying around radish seedlings raised from treated and untreated seed using a range of treatments. In addition, because the treatments themselves may influence fly egg-laying behaviour, seedlings grown from treated seed were re-inoculated with microorganisms and exposed to the flies. In all experiments flies were exposed to plants for 24 h and in each cage a beaker containing only sterilised moist sand was included as a control. A comparison was made of the two types of test cage and of egg-laying around batches of 10-day-old seedlings (10, 40 or 50 seedlings/beaker) and around single radish plants 14 days old.

The number of microbial colonies on batches of test plants was estimated using replicates set aside at the beginning of an experiment. Where single plants were grown in beakers a portion of leaf was removed prior to the experiment for determination of microbial colonies. At the end of an experiment seedlings were counted, weighed and the cabbage root fly eggs extracted using a flotation technique and then counted (Ellis & Hardman, 1975). Egg numbers were transformed to logarithms for analysis.

Results

Effect of treatments on seedling growth and microbial numbers   High concentrations of NaOCl (available chlorine 25 g/l) reduced germination by up to 60%. This necessitated treating larger numbers of seeds to provide sufficient seedlings for experiments. The subsequent growth of these seedlings was unimpaired. All other

Table 1. The effects of different seed treatments on the growth of radish seedlings.

Treatment	Number of seedlings/beaker		Mean weight of seedlings (g)	
	Expt 1	Expt 2	Expt 1	Expt 2
Vancomycin + NaOCl	40	35	0.17	0.15
Streptomycin + NaOCl	39	35	0.17	0.13
Streptomycin + polymyxin + NaOCl	40	—	0.16	—
NaOCl	40	39	0.17	0.13
Untreated	43	35	0.17	0.15

treatments used showed no adverse effects on either seedling emergence or seedling growth (Table 1). None of the treatments consistently eliminated all microorganisms (Table 2) but assessments on 7-day-old seedlings showed that the total number of detectable microbial colonies was reduced from about 10^7 per 5 seedlings for untreated to 0 to 10^6 per 5 seedlings for the treated seeds. The performance of individual treatments varied from occasion to occasion but treatments containing vancomycin and NaOCl or high concentrations of NaOCl alone were the most effective.

Bacteria isolated from radish seedlings were almost all non-sensitive to vancomycin (as determined by tests with antibiotic sensitivity discs) and if this antibiotic was used alone in a seed treatment it was almost without effect on microbial numbers. However, in combination with NaOCl it was highly effective. Treatment with streptomycin alone or in combination with NaOCl exerted considerable selective effects and bacterial colonies surviving streptomycin treatments were streptomycin-resistant. Streptomycin applied on its own was phytotoxic, causing seedling chlorosis, but in combination with NaOCl it was detoxified (Humaydan et al., 1980). Although the results of antibiotic sensitivity tests suggested that combined treatment with streptomycin and polymyxin B should eliminate bacteria from radish seeds, in practice it did not.

Seedlings grown from sterilised seed lacked the characteristic *Brassica* odour

Table 2. The effects of seed treatments on the microbial populations (microbe numbers/5 seedlings) of 7-day-old radish seedlings.

Replicate	Treatment		
	vancomycin + NaOCl	streptomycin + NaOCl	untreated
1	0	1.56×10^6	2.72×10^7
2	0	6.00×10^4	1.80×10^7
3	80	2.28×10^5	1.20×10^6
4	5.76×10^6	4.32×10^5	1.60×10^7

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Table 3. Cabbage root fly egg-laying around radish seedlings raised from treated and untreated seed.

Treatment	Number of eggs/plant		
	Expt 1	Expt 2	Expt 3
Vancomycin + NaOCl	—	3.7	2.2
Vancomycin + thiram + NaOCl	5.1	—	—
Streptomycin + NaOCl	6.8	5.0	2.7
Streptomycin + polymyxin + NaOCl	—	5.1	—
Untreated	12.2	13.3	6.4
Sand control	0.0	0.5	0.0
Least significant ratio ($P = 0.05$)	1.69	2.55	2.17

while plants raised from unsterilised seed had a pungent odour. Plants raised from sterilised seed which were re-inoculated with microorganisms also had a pungent odour.

Effects of treatment on cabbage root fly egg-laying behaviour The various treatments gave the same results in both types of cage. However, flies laid more eggs per plant in the six small cages and so these were chosen for subsequent experiments. In all experiments < 1 egg per beaker was laid on the sand controls and so these results were included in Table 3 but omitted from the analyses. Several treatments were effective in reducing the numbers of eggs laid around plants (Table 3), the most effective being vancomycin and NaOCl which reduced the numbers to less than a third of the number on untreated seedlings. Plants raised from treated seed and then re-inoculated with microorganisms received more eggs than treated plants ($P = 0.05$) but not significantly more than untreated plants (Table 4). Thus, the treatments did not repel the flies.

Table 4. Cabbage root fly egg-laying around radish plants raised from treated seed re-inoculated with microorganisms.

Treatment	Number of eggs/plant
Vancomycin + NaOCl	1.46
Vancomycin + NaOCl + inoculum	5.55
Untreated	3.52
Sand control	0.0
Least significant ratio ($P = 0.05$)	1.92

Table 5. Cabbage root fly egg-laying around individual radish plants raised from treated and untreated seed.

Treatment	Number of eggs/replicate										Geometric mean number of eggs[1]
NaOCl	0	0	4	18	33	47	69	86	88	91	15.5
Untreated	24	24	34	51	73	79	120	190	469	685	92.3

1. Significantly different at $P = 0.05$.

Plant-to-plant variability In all experiments there were large differences in the numbers of eggs laid on individual replicates within treatments. This variation was also reflected in the variable numbers of microorganisms surviving treatments as determined by dilution plating of seedling samples. However, the variation in egg numbers was even greater in untreated replicates and was particularly great in tests involving single radish plants (Table 5). A bacterium was isolated from a number of these single plants which had received large numbers of eggs. This organisms, as yet unidentified, differed in colony morphology from the organisms normally encountered on untreated radish seedlings.

Discussion

To eliminate microorganisms from seed is extremely difficult without damaging plant tissues. Hough et al. (1981) showed that treatment of onion seeds with a combination of vancomycin, thiram and NaOCl eliminated microorganisms from approximately 90% of seeds. In the present study it was also shown that although no treatment consistently eliminated microorganisms from all radish seeds, there was a considerable reduction in the total numbers of microorganisms present. Moreover, with streptomycin treatments, besides a reduction in microorganisms there was a change in the components of the microflora.

There was considerable variation in the numbers of eggs laid on the replicates of the various treatments and much of the effort in this study was devoted to improving the control of microorganisms and increasing the uniformity of the treated plants in respect of their microflora. However, up to now little attention has been paid to the even greater variation which was apparent in both the microbial population and the numbers of eggs laid on untreated replicates. The flies' consistent preference for individual radish plants was also observed in earlier studies (Ellis & Hardman, 1975). It is possible that the extremely high preference shown for certain untreated plants was due to the presence of specific stimulatory organisms not generally distributed on all seedlings. Further study of these stimulatory organisms should show whether it is possible to manipulate the microflora through seed inoculation to establish less attractive microbial populations. The study of this possibly stimulatory microorganisms may also reveal information on host plant volatiles which could be used in the development of more efficient traps for cabbage root fly. If manipulation of the microflora colonising radish seed could be achieved in a field

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situation it may be possible to reduce cabbage root fly attack. Similar studies have shown that bean seed fly damage to snap beans in the field can be reduced by seed treatments which alter the plant's microflora (Harman et al., 1978); a type of biological control may therefore be developed from this work. However, radish plants would require protection from cabbage root fly for a longer period than beans which are particularly attractive to bean seed fly as seedlings. This study has revealed that the complex relationship which exists between an insect and its host plant is one which also involves microorganisms. The information not only increases our knowledge of the biology of cabbage root fly but may also be of value in studies of other hosts of this pest and of other pests of crucifers which locate their hosts through a response to plant volatiles.

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Activity of cabbage extracts in deterring oviposition by the cabbage looper, Trichoplusia ni

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Abstract

Macerated cabbage tissue sprayed onto intact cabbage plants deterred oviposition by *Trichoplusia ni* for a period of at least 8 days under laboratory conditions. Steam distillates of cabbage leaves were deterrent on the first night after application to plants, but the activity disappeared after 24 h. Observations on ovipositing moths indicated that more contacts were made with untreated plants than with plants sprayed with cabbage macerate. Also, more contacts resulted in oviposition on the untreated plants. The results suggest that the deterence involves the combination of volatile and non-volatile components. The active material is highly soluble in diethylether and can be separated into two major fractions by column chromatography on Florisil.

Key-words: Trichoplusia ni, cabbage macerate, oviposition deterrents, repellents

The cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) feeds on a wide range of host plants, many of which are important crops. The ovipostion behaviour of this insect is mediated to a large degree by the presence or absence of larvae on potential host plants (Renwick & Radke, 1980). The deterrent effect of larval feeding probably functions to ensure even distribution of the population over available food resources. Similar spacing mechanisms have recently been described for several phytophagous insects (Prokopy, 1981).

The chemicals responsible for deterring ovipostion by *T. ni* are present in the larval frass and in disrupted tissue of host plants (Renwick & Radke, 1980; 1981). We now report the results of investigations into the nature of the deterrent, the behavioural response of gravid females, and the development of a scheme for chemical isolation of the active materials.

Materials and methods

Cabbage looper adults were obtained from a laboratory colony and used in oviposition assays as previously described (Renwick & Radke, 1981). A second colony established from field-collected pupae (in Tifton, Georgia) was confined to cabbage plants for oviposition and larval feeding. Moths from this colony laid most of their eggs on the test plants, whereas moths from the diet-reared laboratory colony

laid a large proportion of their eggs on the sides of the cages. The new colony was therefore used in experiments to observe the oviposition behaviour in response to a choice of treated and untreated plants. In each experiment observations were made in two pairs of moths in a 48 × 48 × 48 cm bioassay cage in subdued light during the first 90 min of the dark period.

Cabbage plants (var. Golden Acre) were grown in a greenhouse with supplementary lighting. The plants were used in bioassays about 6 weeks after planting and for macerate or extract preparation after 8 weeks. Macerated tissue was prepared in a Waring blendor and applied to test plants at two concentrations: 20 g fresh weight in 100 ml water for observation experiments and 100 g in 100 ml for studies on the duration of the deterrent effect. Enough plants were treated at the same time for testing on eight consecutive nights. Solvent extracts and fractions were applied to the plants in diethylether solution. When 15 ml of either was used in a fine spray, the solvent evaporated quickly, with no visible damage to the plant. Control plants in choice bioassays were sprayed with ether or water as appropriate. Steam distillation over a period of 2 h was carried out with intact cabbage leaves. The distillate was extracted with ether, and the volume adjusted to provide an extract equivalent to 100 g tissue in 100 ml ether. This extract was applied to enough plants for assays on two consecutive nights.

Extraction of cabbage for chemical isolation of the deterrent was performed by dropping the leaves into boiling ethanol, homogenizing in a Waring blendor, and filtering through glass wool. The ethanol extract was evaporated to dryness for determination of solubility in various solvents and subsequent column chromatography on Florisil.

Bioassay results were expressed as percent deterrence, obtained by dividing the difference in number of eggs on control and treated plants by the number on the control plant.

Results

When the deterrent effect of macerated cabbage at a concentration of 100 g fresh weight in 100 ml was followed over a period of 8 days, no loss of activity was observed (Table 1). The deterrence remained above 90% for the whole period.

Observations on the behaviour of individual moths were designed to determine the mechanism of avoidance or rejection of treated plants. The number of times each female made contact with either a control or treated plant was recorded, and whether or not oviposition occurred was noted. In a series of three experiments, a total of 348 contacts were made with the control plants and only 52 with the treated plants (Table 2). Eighty-six percent of the contacts with control plants resulted in deposition of an egg, whereas only 25% of the contacts with the treated plants were followed by oviposition.

The possible involvement of volatile plant constituents in the oviposition deterrence of *T. ni* was investigated by testing steam distillates of cabbage tissue. Plants treated with an ether solution of the steam distillate were avoided on the first day. However, treated plants tested on the second day were no longer significantly deterrent (Table 3).

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Table 1. Effect of cabbage macerate on oviposition by *Trichoplusia ni*.

Day	Number of eggs control	Number of eggs treated	Deterrence (%)
1	202	7	97
2	63	2	97
3	89	1	99
4	170	8	95
7	302	2	99
8	226	5	98

Table 2. Contact and oviposition by *Trichoplusia ni* on cabbage plants treated with cabbage macerate.

Experiment	Treated number of contacts	Treated number of eggs	Control number of contacts	Control number of eggs
1	11	4	99	94
2	12	1	152	141
3	29	8	97	64
% contacts resulting in oviposition	25		86	

Table 3. Effect of cabbage steam distillate on oviposition by *Trichoplusia ni*.

Experiment	Day 1 treated	Day 1 control	Day 1 deterrence (%)	Day 2 treated	Day 2 control	Day 2 deterrence (%)
1	43	217	80	57	53	-
2	16	31	48	67	62	-
3	10	73	86	65	63	-
4	20	189	89	57	52	-
5	7	71	90	27	21	-
6	25	487	95	33	121	72
7	56	672	92	152	249	39
8	22	409	95	227	128	-
9	15	328	95	132	182	27
Total	214	2477		817	931	
$\bar{x}$	24[1]	275	91	91	103	12

1. Significantly different from control at $P \leqslant 0.005$ (Mann-Whitney U-test).

Preliminary work on the isolation of the deterrent has shown that the active material can be extracted into hot ethanol. After evaporation of the ethanol, several solvents were tested for solubility of the active residue. Diethylether appeared to be particularly effective. Solubility in non-polar solvents was limited, but repeated washings with hexane removed all the activity, and hexane solutions were used for applications to a Florisil column. Step-wise elution of the material from Florisil was obtained with hexane, chloroform, methyl-tert butyl ether (MTBE) and methanol. Active fractions were obtained in the chloroform and methanol, with some lower activity occurring in a fraction eluting with MTBE.

Discussion

The results clearly show that the deterrent effect of macerated cabbage tissue on oviposition by *T. ni* persists for a period of at least 8 days in the laboratory. Thus a major component of the deterrent must be relatively stable and non-volatile. Observations on ovipositing moths, however, indicated a bimodal mechanism of deterrence. The moths appeared to avoid treated plants to a large extent. This behaviour would suggest the involvement of volatile material. But the lower frequency of oviposition following contact with treated plants would indicate rejection in response to a contact stimulus. The involvement of a volatile component was further suggested by the results of bioassays of steam distillates of cabbage tissue. The high level of activity obtained with these volatile extracts quickly disappeared after 1 day.

The lasting effect of chemicals responsible for spacing of phytophagous insects appears to be rather general. Schoonhoven et al. (1981) found that the oviposition-deterrent pheromone associated with eggs of *Pieris brassicae* retains its activity for at least 7 weeks, and plants treated with deterrent extracts remain deterrent for at least 14 days. The marking pheromone of the apple maggot fly, *Rhagoletis pomonella*, persists for at least 4 days (Prokopy, 1972), and the oviposition deterrent of the sorghum shoot fly, *Atherigona soccata*, is active for at least 5 days (Raina, 1981). Examples of volatile oviposition deterrents are less common. However, the signal associated with an egg of the moth *Hadena bicruris* in a *Melandrium* flower lasts for only 1 day (Brantjes, 1976), and volatiles released from damaged corn plants deter oviposition by the European corn borer, *Ostrinia nubilalis* (Schurr & Holdaway, 1970). The nature of the message to be conveyed is likely to determine whether volatile or non-volatile chemicals are utilized by the insects. In most cases, the signal of occupancy must persist until other cues are operative. The short duration of the *Hadena bicruris* deterrent is sufficient, however, since a preference exists for 1 day-old flowers (Brantjes, 1976). The involvement of volatiles in deterring oviposition by *T. ni* may serve to emphasize the unsuitability of a plant when larvae are actively feeding.

Our preliminary chemical studies on the *T. ni* deterrent indicate that at least two compounds are involved. Both are highly soluble in ether, but are well separated by Florisil chromatography. The non-polar nature of the active material may be typical of the deterrents of plant origin. The olive fruit fly, *Dacus oleae*, uses juice from the oviposition punctures to mark occupied fruits (Cirio, 1971), and the deterrent com-

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pounds appear to be relatively non-polar (Vita, 1978; Girolami et al., 1981). However, the pheromones associated with oviposition by *Rhagoletis pomonella* (Prokopy, 1972) and *Pieris brassicae* (Rothschild & Schoonhoven, 1977) are water soluble. The only reported case of a non-polar oviposition-deterring pheromone in a phytophagous insect appears to be that of the azuki bean weevil, *Callosobruchus chinensis* (Oshima et al., 1973).

We can conclude that the material in disrupted cabbage tissue that deters oviposition by *T. ni* consists of both volatile and nonvolatile components. The deterrent activity remains for several days and is soluble in organic solvents. This property might be particularly advantageous in terms of pest management, since the chemicals would not be readily washed from the plants by rain. The protection qualities of extracts will be tested in the field and chemical studies are presently underway to isolate the active compounds.

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Perception of the oviposition deterrent pheromone in Pieris brassicae

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Abstract

Egg-laying behaviour of female *Pieris brassicae* was studied in the laboratory. The activity of various concentrations of egg wash containing the oviposition deterrent pheromone, was determined in choice experiments with cabbage leaves. A detailed description of the oviposition behaviour indicates three chemoreceptory systems possibly involved in the perception of the pheromone. Besides antennal olfactory hairs, gustatory hairs also seem to be involved. Single cell recordings from tarsal taste hairs suggest the presence of one particular sense cell responding to one or more components in the egg wash. The presence of taste hairs on the ovipositor was demonstrated by electrophysiological and SEM techniques. However their importance in the perception of the pheromone remains questionable.

Key-words: ovipositor, chemoreception, *Pieris brassicae*, oviposition deterrents, gustatory receptors

The presence of conspecific eggs plays an important role in the oviposition behaviour of *Pieris brassicae* (Lepidoptera: Pieridae). The butterflies prefer 'clean' leaves to leaves already carrying conspecific eggs (Rothschild & Schoonhoven, 1977). Besides the colour of eggs, a pheromone-like emanation associated with the eggs was found to be responsible for the deterrent effect. Furthermore, leaves from which eggs had been removed were still deterrent. Olfactory hairs on female antennae can perceive the odour of eggs (Behan & Schoonhoven, 1978), although as yet no specifically tuned cells or groups of cells were found (den Otter et al., 1979).

In cooperation with the Netherlands Organization for Applied Scientific Research TNO, the elucidation of the structure of the deterrent compound(s) is being attempted. The present paper gives some details on the bioassay, egg-laying behaviour and physiological responses of some chemoreceptors which are likely to be involved in oviposition.

Bioassay

Pieris brassicae females were obtained from a colony maintained for several generations on cabbage plants (*Brassica oleracea* var. *gemmifera*). Rearing conditions
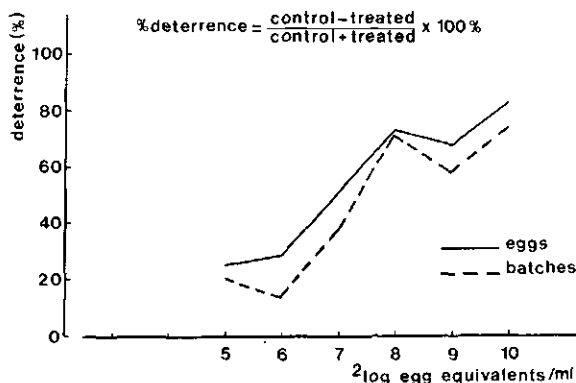
Fig. 1. Deterrent activity of egg wash (at concentrations 2^5-2^{10} egg equivalents/ml on oviposition by *Pieris brassicae* females.

were similar to those described by David & Gardiner (1952). The bioassay for detecting the pheromone is based on choice experiments with matched pairs of cabbage leaves, taken from the same plant. Pheromone solutions were prepared by washing eggs for 5 min in pure methanol. Various concentrations of egg wash, ranging from 32-1024 (2^5-2^{10}) egg equivalents per ml (ee/ml) were used. One leaf of a pair was sprayed with 1 ml of egg wash (treated leaf) and the other leaf with 1 ml of solvent (control leaf). Trials were conducted in cages (80 $\times$ 100 $\times$ 80 cm), containing groups of male and female butterflies, illuminated by a mercury vapour lamp and additional natural light. Experimental animals varied in age from 4-14 days after emergence. The leaves were arranged with their petioles in water and were exposed simultaneously to the butterflies for one hour. The egg batches and eggs laid on both leaves were then counted and the percentage deterrence was calculated. The number of trials of concentrations of 2^5, 2^6, 2^7, 2^8, 2^9 and 2^{10} ee/ml was 19, 16, 37, 30, 20 and 12 respectively (see Figure 1).

Responses to various concentrations of egg wash are shown in Figure 1. Percentage deterrence, for both total numbers of eggs and of batches, increases with the concentration of egg wash. At all concentrations tested, except for 64 ee/ml (2^6), the numbers of eggs laid on treated leaves were significantly lower than those on control leaves (Wilcoxon matched-pairs signed-ranks test, $P < 0.01$). This means that, at least under laboratory conditions, females can detect relatively small amounts of pheromone. On the other hand, a high concentration of pheromone does not suppress oviposition completely. As long as we do not know the chemical structure and properties of the deterrent compound(s), the actual concentration present on and around the leaf, and thus perceptible for the female, remains unknown. The concentration units used here have only relative value and they are difficult to compare with actual numbers of eggs.

Behaviour

Based on observations on the oviposition behaviour in the laboratory, several be-

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havioural steps or actions can be distinguished. Usually these steps are displayed in the sequence given in Table 1. The third action in this sequence is the 'drumming behaviour', described previously by Ilse (1937) and Terofal (1965). Most of the time it follows immediately after 'landing' and sometimes it is continued during 'curving' and 'touching', the next two steps in the behavioural chain. The biological significance of the tapping movements of the fore tarsi could be enhancement of the perception of nonvolatile chemicals present in the leaf (Ma & Schoonhoven, 1973). Furthermore, 'drumming behaviour' is always accompanied by fluttering movements of the wings which may be interpreted in either of two ways. Firstly, they might serve to compensate for a certain loss of grip during 'drumming'. Secondly, the wing movements might evoke an airstream prompting the perception of volatile substances, such as the pheromone and leaf chemicals. Fluttering could have a duel function. Another phenomenon worth mentioning is the observation that several females break off the regular sequence after bending the abdomen ('curving') but before contact has been made with the lower leaf surface ('touching'). Possibly additional information from abdominal chemoreceptors is taken into account in the decision of the female whether or not to oviposit. The ethogram (Table 1) suggests three behavioural levels at which the oviposition deterrent pheromone might operate. Firstly, during approach flight (antennal olfactory hairs); secondly, during 'landing' and 'drumming' (tarsal taste hairs) and thirdly, during 'curving' and 'touching' (abdominal taste hairs).

Table 1. Qualitative description (ethogram) of egg-laying behaviour of *Pieris brassicae* females in the laboratory.

|		Senses (possibly) involved
Approach	♀ flies in rather straight line to the leaf, sometimes interrupted by 'turning' i.e. a sudden change in flight-direction (away from the leaf)	vision olfaction
Landing	the first contact with the leaf by the-tarsi, often immediately followed by	(olfaction) tarsal tastehairs
Drumming	alternate tapping movements on upper leaf surface with fore-tarsi	(olfaction) tarsal tastehairs
Curving	♀ bends her abdomen around the edge of the leaf without touching lower surface	tastehairs on tarsi and ovipositor
Touching	touching lower leaf surface with the ovipositor	tastehairs on tarsi and ovipositor mechanoreceptors on ovipositor
Oviposition	deposition of the eggs, one by one, in a badge	mechanoreceptors and tastehairs on ovipositor

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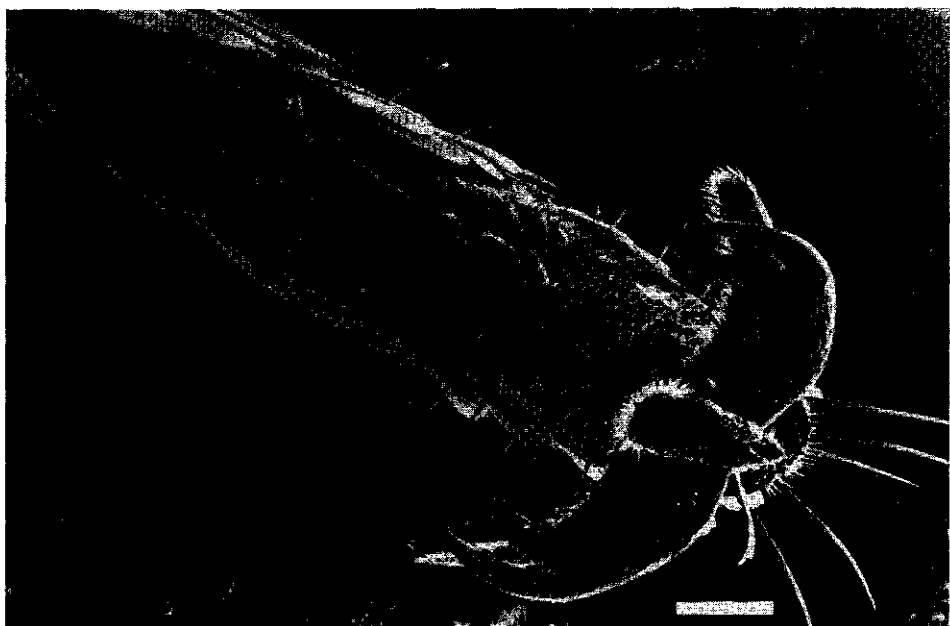
Fig. 2. Distal part of female fore-tarsus. Arrow indicates one group of taste hairs on the 5th tarsomere. Bar = 100 μm.

Chemoreceptors

The olfactory hairs have been investigated by Behan & Schoonhoven (1978) and den Otter et al. (1979). Therefore this discussion will be restricted to the gustatory hairs. Ma & Schoonhoven (1973) found the so-called B-hairs on the fifth tarsomere of female fore-tarsi (Figure 2) to be taste hairs with five receptor cells. Responses to salts and various glucosinolates indicated the presence of two salt cells and one glucosinolate-cell. In addition, a mechanoreceptor cell was found. The fifth sense cell demonstrated in morphological studies could not be identified electrophysiologically. In our experiments, single cell recordings were made employing the methods described by Ma & Schoonhoven (1973). As stimuli, various concentrations of egg wash and 20 mmol/l glucotropaeolin (a glucosinolate) were used. Sodium chloride (50 mmol/l) was added to all stimuli and served as a control stimulus. A representative recording is shown in Figure 3. Sometimes two cells can be observed responding to salt but in the particular recording of Figure 4 (upper trace) only the response of one spike can be seen. The middle trace represents the response to egg wash (1000 ee/ml), showing a marked increase in spike frequency during the first second of stimulation. These spikes merely originate from one of the so-called salt-cells. Such an increase of spike frequency during stimulation with egg wash was found in more than 70% of the recordings. Also glucotropaeolin evokes a marked increase in spike frequency (Figure 3, lower trace). These spikes, however, could be
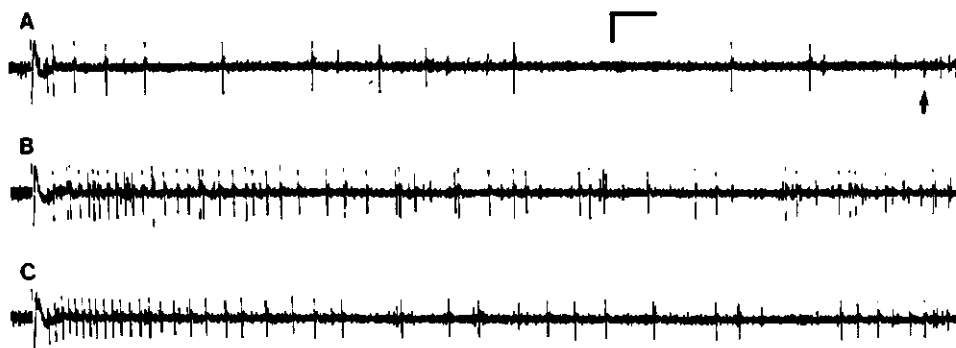
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Fig. 3. Responses of one tarsal taste hair to (A) 0.05 mol/l NaCl, (B) egg wash (1000 ee/ml), and (C) 0.02 mol/l glucotropaeolin. Arrow indicates bending of the hair, showing stimulation of the mechanoreceptor. Calibration: 0.5 mV; 50 ms.

demonstrated to originate from the glucosinolate-cell. Thus the assumption made by Behan & Schoonhoven (1978) that contact discrimination is involved in the detection of the oviposition deterrent pheromone is corroborated by our finding of a particular cell responding to egg wash. This means that relatively non-volatile components also occur in the pheromone.

The presence of chemoreceptors on the ovipositor, as suggested by behavioural observations was confirmed by scanning electron microscope studies. The caudal view at the ovipositor (Figure 4A) shows two groups of 11-15 hairs located near the edges of the lateral valves of the oviduct. These hairs are placed on sockets (Figure 4B), are rather short (about 90 μm) in comparison to surrounding hairs and possess a blunt tip with a pore-like structure (Figure 4C). Responses of these hairs to egg wash (500 ee/ml) show a slight, but significant increase in total spike frequency as compared to the salt response (Figure 5). (Wilcoxon matched-pairs signed-ranks test, $P < 0.01$). This increase cannot be ascribed to increased activity of one particular sense cell. As yet, we have not identified the number of chemosensory cells present in these hairs, which also have a mechanoreceptor. Stimulation with solutions of glucosinolates evokes responses which do not differ from control (salt) stimulations. Therefore, it seems unlikely that these hairs are involved in hostplant recognition. Whether or not they play a role in the perception of the oviposition deterrent pheromone remains unclear. Their responses to egg wash are quite low, but the summed responses of all 22-30 hairs present on the ovipositor could very well lead to significant changes in the behavioural chain.
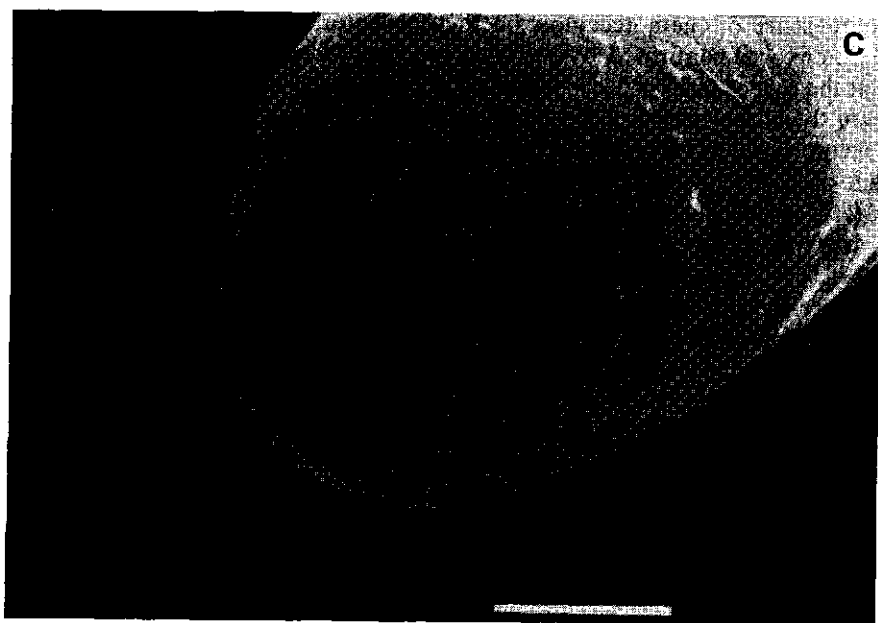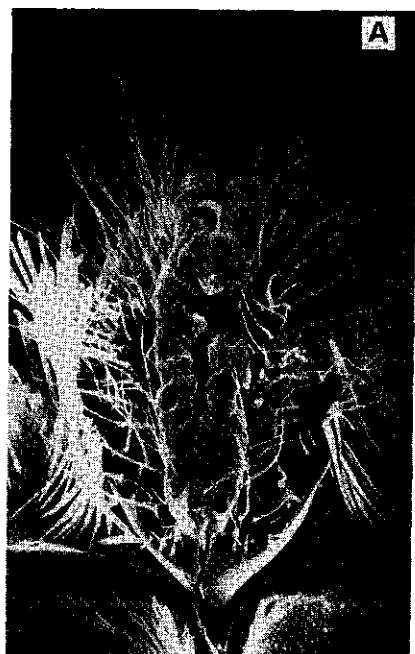
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Fig. 4. Abdominal taste hairs of female *Pieris brassicae*: (A) caudal view at the ovipositor, arrow indicates one group of taste hairs; (B) details of one group of taste hairs; (C) top structure of one abdominal taste hair, bar = 1 μm.

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Fig. 5. Responses of one abdominal taste hair to (A) 0.05 mol/l NaCl, and (B) egg wash (500 ee/ml). Arrow indicates bending of the hair, which evokes activity of the mechanoreceptor. Calibration: 0.05 mV; 50 ms.

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Interference with the establishment of the leafhopper Amrasca devastans on its host plants by certain non-host plants

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Abstract

This paper gives the effects of the non-hosts castor and sponge gourd on certain responses of *Amrasca devastans* to cultivars of its host: highly succeptible cotton *Gossypium hirsutum* cv. PS-10 and moderately resistant cotton cv. H-14. The arrival and stay of the adult females on the host leaves were significantly reduced in the presence of the leaves of sponge gourd but not of castor. The oviposition by the insects on each host was also reduced in the presence of each non-host tested, the reduction in the number of eggs laid being about 80-85%. The total number of eggs laid on both the host and non-host leaves, when presented together, was also considerably reduced with castor but not with sponge gourd because the latter itself elicited adequate oviposition. However, the nymphs emerging from the eggs laid on the gourd leaves failed to develop on them and died. The reduction in the number of eggs laid on the host by the gourd leaves was due to their drawing away more insects than the host and not by their volatiles which, in fact, increased the egg-laying. On the other hand, the decline in the number of eggs laid on the host in the presence of the non-host castor was caused by the volatiles of the latter. These observations suggest the possibility of interfering with the establishment of the leafhopper for its management by reducing arrival, stay and/or ovipositional responses of the insects to their hosts by combining them with appropriate non-hosts as intercrops.

Key-words: Amrasca devastans, oviposition, mixed cropping, cotton, plant odours

The establishment of the cotton leafhopper *Amrasca devastans* (Distant) (Homoptera: Cicadellidae) on different plants is determined primarily by the following responses (Saxena et al., 1974):
1. orientation, determining its arrival on the plants
2. feeding
3. utilisation of ingested food, determining the insect's nutrition
4. development
5. egg production
6. oviposition

Responses 1, 2 and 6 are involved in the behavioural selection or rejection of a plant whereas nutrition plays its role in the insect's development and egg production

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after it arrives and feeds on a plant. These behavioural responses are determined not only by sensory stimuli from the plants but also by their interaction with stimuli from the background (Saxena & Saxena, 1974, 1975a, 1975b). However, we have little knowledge of how various non-host plants in the vicinity of the hosts would interact with the latter to determine the establishment of the leafhopper. If such an interaction can interfere with the insect's establishment on the hosts it may be possible to use the non-hosts concerned as intercrops for the management of the pest. To investigate this possibility, various non-host plants of the insect are being screened in the laboratory in small arenas under controlled conditions to find those promising species which can be subsequently tested in the fields.

The non-hosts being examined are those which are occasionally grown as intercrops with different hosts by farmers. The responses of the leafhopper being studied are: arrival and stay, oviposition and development on the leaves of the test plants. The results of our study of the interaction of two non-hosts, namely, castor (*Ricinus communis* L.; Euphorbiaceae) and sponge gourd (*Luffa cylindrica* (L.) Roem.; Cucurbitaceae) with two hosts, namely, the highly susceptible cotton *Gossypium hirsutum* L. cv. PS-10 and the moderately susceptible cotton cv. H-14, are presented here.

Materials and methods

Field-collected nymphs of *Amrasca devastans* (Distant) were reared on unripe fruits of okra (*Abelmoschus esculentus* L. (Moench)) (Saxena & Saxena, 1971). The emerging males and females were kept in pairs on the same food source until they were 10 days old when they show maximum egg-laying (Saxena & Saxena, 1971). These females as well as second instar nymphs, were tested for various responses as described below.

Each test was conducted with at least 50 individuals arranged in 5 replicates of 10 each. The responses of the insects under different conditions were statistically processed and compared by appropriate procedures (Snedecor & Cochran, 1967), involving analysis of variance (ANOVA), followed by F-test which, if significant, was followed by computation of the least significant difference (l.s.d.) between the mean responses under comparison.

The plants tested were 4-8 weeks old. Various tests were conducted in a cylindrical chamber of clear plastic or in a rectangular chamber of plexiglass. The cylindrical chamber consisted of two removable compartments, one above the other, each being 10 cm high and 8.5 cm in diameter. The upper compartment had a removable cover and a fixed bottom of nylon net resting on the top open end of the lower compartment having a plastic bottom. A freshly excised leaf of the test plant was trimmed to form a rectangle (5 × 3 cm) and its 2-centimetre petiole was immersed in water in a vial (3.5 cm diam.; 2.5 cm ht.). One or two such rectangular leaves of the same or different plants were placed in the upper compartment. For certain tests, 10 g freshly excised leaves of the test plants were kept in the lower compartment so that their volatiles could diffuse into the upper compartment bearing the leaf rectangle (Saxena & Basit, 1982).

Ten insects were introduced into the chamber for 24 h or more under a 13-hour

photophase. For studying their arrival and stay on the leaves, the test chambers were placed under a uniform illumination from above by a 40-watt daylight fluorescent tube and the percentage of females present on each leaf after 1, 2 and 24 h was recorded. For ovipositional tests, the leaves were removed from the chamber and treated with lactophenol (Garlson & Hibbs, 1962; Saxena & Basit, 1982) to reveal the eggs which were counted. For studying the development, the percentage of second instar nymphs developing to the adult stage and the period for doing so were recorded.

The rectangular plexiglass chamber (40 × 20 × 20 cm) was used to provide the above mentioned leaves in 4 rows (6 cm apart) of 3 stations (3 cm apart) each. In some experiments, all 4 rows were of the host leaves, in others 2 rows of the host alternated with 2 of the non-host leaves. Ten females were introduced into the chamber for 24 h and the numbers of eggs laid by them on the leaves were recorded as described above.

Results

Arrival and stay on the leaves of the plants An insect must first arrive and stay on a plant before it can feed or oviposit on it for its establishment. The leafhopper *A. devastans* has been shown before to arrive and stay on certain hosts as well as non-hosts as a result of its orientational responses involving its attraction and arrest by their various stimuli (Saxena et al., 1974; Saxena & Saxena, 1974, 1975a, 1975b). The unspecific visual (green colour) and hygrostimuli from the hosts as well as non-hosts elicit a fairly high attraction and arrest while the specific olfactory stimuli from the hosts enhance these responses of the leafhopper.

The present work shows that the colonisation by ovipositing females of single host or non-host leaves increased with time after the start of the experiment (Figure 1). The percentages of insects on the leaves of each host were very high after 24 h (NIL - 1 - H), those on the non-host sponge gourd were equally high (LC - 1 - NH) and those on the non-host castor were about one-third less (RC - 1 - NH).

On presenting two leaves of the same host, the total percentage of insects on the two leaves was almost equal to that on one leaf alone (Figure 1: NIL - 2 - H_1, H_2) but each leaf only had about half the number. When a host leaf was combined with a leaf of the non-host castor, the percentage of insects on both was again almost equal (RC - 2 - H, NH). Thus, castor leaves did not affect the arrival and stay of the insects on the host leaves when present together though the responses to castor leaves were lower when given alone. On the other hand, combining a host leaf with that of the non-host sponge gourd resulted in a significantly higher percentage of insects colonising the latter (LC - 2 - NH). Thus, this non-host interfered with the arrival/stay of the insects on the host leaf by drawing them away to itself. This pattern of responses was similar with both the cotton varieties.

Ovipositional responses When a single leaf of any test plant was given, the number of eggs laid by the females was greatest on the highly susceptible cotton cv. PS-10 (Figure 2B: 1 - H), about half as much on the moderately susceptible cotton cv. H-14 (Figure 2A: 1 - H) and on the non-host sponge gourd (Figure 2A and B: LC -
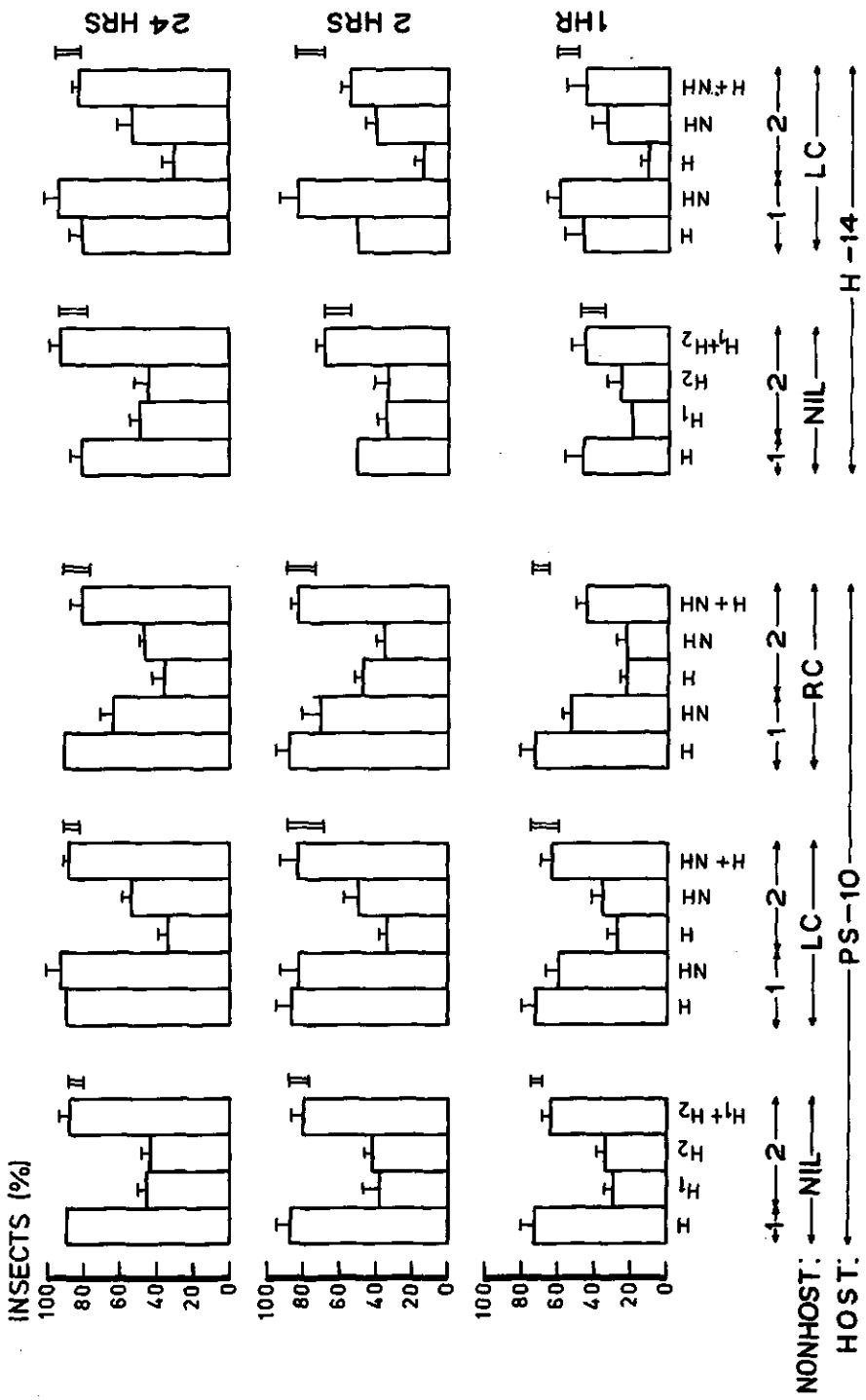
1 - NH), and extremely low on the non-host castor (Figure 2A and B: RC - 1 - NH).

On presenting 2 leaves of either of the hosts, the number of eggs laid on each (Figure 2A and B: NIL - 2 - H_1, H_2) was about three-fourths that on the same leaf presented alone (Figure 2A and B: NIL - 1 - H), so the total number of eggs on both the stations of each host was higher (Figure 2A and B: NIL - 2 - ($H_1 + H_2$)) than on a single leaf. When a leaf of a test host and a leaf of a non-host were combined, the number of eggs laid on the former declined to about one-fourth (Figure 2B: LC - 2 - H, RC - 2 - H) to one-sixth (Figure 2A: LC - 2 - H, RC - 2 - H) of that on the same host leaf presented alone. But, the number of eggs laid on the gourd leaf in these tests remained as high (Figure 2A and B: LC - 2 - NH) and on the castor leaf as low (Figure 2A and B: RC - 2 - NH) as that on the same leaves given alone. The total number of eggs on both leaves was about one-eighth (Figure 2A: RC - 2 - (H + NH)) to one-fourth (Figure 2B: RC - 2 - (H + NH)) of that on the host leaves alone when the non-host was castor. On the other hand, on combining the non-host gourd with the host cotton cv. PS-10, the total number of eggs on the two leaves declined to about three-fourths of that on the host leaf in single-station tests (Figure 2B: LC - 2 - (H + NH)). No such decline was observed by combining the gourd leaf with that of the host cotton cv. H-14 (Figure 2A: LC - 2 - (H + NH).

In order to examine whether the above mentioned reduction in egg-laying would be obtained if the host and non-host leaves were available at multiple stations in a larger arena, the tests were conducted in the rectangular plexiglass chamber. When 6 leaves of only the host cotton cv. H-14 were available in 2 rows, the total number of eggs (Figure 2C: a - H(2)) was almost the same as that on 12 leaves in 4 rows of the same host (Figure 2C: a - H(4)). But, on alternating 2 rows of the leaves of the non-host sponge gourd with 2 rows of the host cotton cv. H-14 (Figure 2C: b) the number of eggs laid on the latter was reduced to about one-tenth and the total number on both the host as well as the non-host to about one-half of those laid on the host leaves presented alone (Figure 2C: a).

To see if the reduction in the number of eggs laid on the host leaves in the presence of the non-host leaves was caused by their volatiles, the insects were offered single leaf rectangles of the host cotton cv. PS-10 in the upper compartment of the cylindrical chamber permeated with the vapours of the leaves of different plants in the lower compartment. The number of eggs laid on the host leaf was significantly

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Fig. 1. Presence of *Amrasca devastans* females on leaves of certain host and non-host plants at 1, 2 and 24 h. Single vertical bars represent standard errors, double vertical bars l.s.d. values at $P = 0.05$.
H: responses to 1 host leaf presented alone or with a non-host leaf.
H_1, H_2: responses to 2 host leaves presented together.
NH: responses to 1 non-host leaf presented alone or with a host leaf.
H + NH: sum of responses to host and non-host leaves presented together.
$H_1 + H_2$: sum of responses to 2 host leaves presented together.
1, 2: Number of leaves presented per test.
LC: *Luffa cylindrica*; RC: *Ricinus communis*; H-14: *Gossypium hirsutum* cv. H-14; PS-10: *G. hirsutum* cv. PS-10.

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increased by the volatiles of the leaves of the same host or of the non-host sponge gourd (Figure 3). But, the volatiles of the non-host castor significantly reduced the number of eggs to about one-half of that in the control.

Nymphal development Since the leafhopper laid eggs on the leaves of the non-host sponge gourd, it was necessary to examine if the emerging nymphs would complete development on this plant and compare it with the other test plants. The percentage of insects completing development was very high on the host cotton cv. PS-10, about one-half as much on the cotton cv. H-14, one third on the non-host gourd and nil on castor (Figure 4), the period of development ranging between 5.5 and 8.0 days.

Discussion

This work shows that certain steps in the establishment of an insect pest involving its arrival/stay, oviposition and development on its host plants can be interfered with by some of its non-hosts present in the vicinity. With particular reference to the cotton leafhopper *Amrasca devastans*, the leaves of the two non-hosts tested in this work have been shown to reduce the number of eggs laid by the insect in one of the following ways:

– A reduction in the number of eggs laid on the host leaves without a reduction in the egg-laying on the non-host leaves nor in the total number of eggs laid e.g., cotton cv. H-14 combined with the sponge gourd.

– A reduction in the number of eggs laid on the host leaves as well as in the total number of eggs on the leaves of both the plants but the egg-laying on the non-host remaining normal e.g., cotton cv. PS-10 combined with the sponge gourd.

– A reduction in the number of eggs laid on the host leaves as well as in the total number of eggs on the leaves of both the plants together, the egg-laying on the non-host remaining very low e.g., cotton cv. PS-10 or H-14 combined with castor.

The establishment of certain other insect species on their host plants has been reported to be interfered with by certain non-host plants. Such an interference with the establishment of the flea beetle *Phyllotreta cruciferae* on collards by tomato plants is stated to be through the latter's olfactory stimuli (Tahvanainen & Root, 1972). But, no olfactory interference could be detected for the reduction in oviposition by *Plutella xylostella* and *Aleurodes brassicae* on their hosts Brussels sprouts by the non-host tomato (Perrin & Phillips, 1978). The present work, however, demon-

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Fig. 2. Oviposition by 10 *Amrasca devastans* females in 24 h on host and non-host leaves presented alone or together. Abscissa in A and B as in Figure 1.
Abscissa in C:
H(2): responses to 6 host leaves presented in 2 rows.
H(4): responses to 12 host leaves presented in 4 rows.
NH(2): responses to 6 non-host leaves presented in 2 rows each alternating with 2 rows of host leaves.
H(2)+NH(2): sum of responses to host and non-host leaves presented in 2 rows each alternating with one another.
Other legends as in Figure 1.
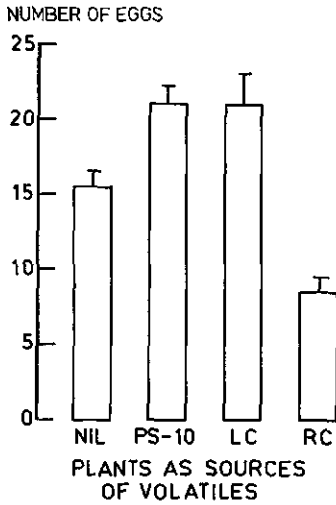
NUMBER OF EGGS

Fig. 3. Effects of volatiles of host and non-host plants on oviposition by 10 *Amrasca devastans* females in 24 h on leaves of susceptible cotton cv. PS-10.
Other legends as in Figure 1.

strates that the reduction in the number of eggs laid by the leafhopper on its host leaves is caused by one of the following:
− A reduction in the proportion of insects colonising the host leaves as by the non-host sponge gourd the volatiles of which enhance the insect's ovipositional response.
− A reduction in egg deposition by the insects after their arrival on the host leaves by the volatiles of the non-host castor leaves which do not affect the arrival/stay of the insects on the host leaves.

Fig. 4. Development of nymphs of *Amrasca devastans* on host and non-host plants.
Other legends as in Figure 1.

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These observations provide a basis for developing two approaches to utilising appropriate non-host plants of the leafhopper to interfere with its establishment for its management. First, the insect's arrival/stay and oviposition can be reduced by c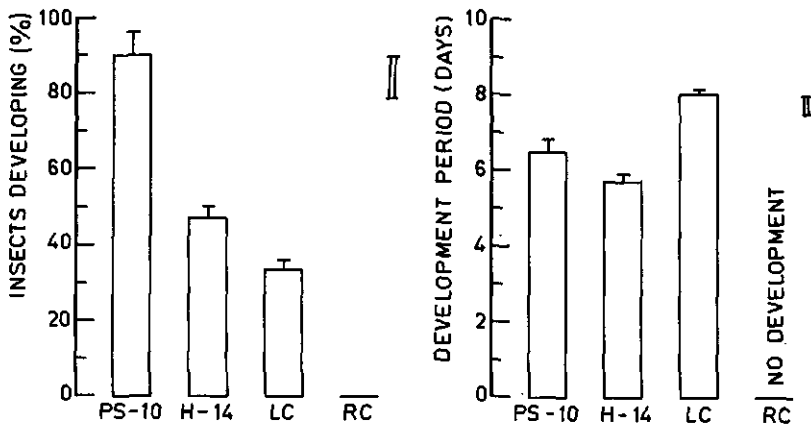ombining the host plants with appropriate non-hosts as intercrops which are compatible and economically important. The non-host suitable for the purpose may belong to one of the two categories:

— Those non-hosts e.g., castor, which do not elicit oviposition on themselves and reduce the number of eggs laid on the hosts as well as the total number of eggs laid on both the plants.

— Those non-hosts e.g., sponge gourd, which elicit oviposition on themselves but reduce the number of eggs laid on the hosts without reducing the total number of eggs on both the plants. The eggs laid on such non-hosts would hatch and the emerging nymphs, if able to develop, would contribute to the build-up of the pest's population. But, since the sponge gourd leaves do not support development of the leafhopper, this non-host can also serve as an intercrop for interfering with the pest's establishment.

The second approach involves inhibition of oviposition by the leafhopper on its host plants by permeating the surrounding air with volatiles of appropriate non-hosts suitably extracted and dispended, as considered before (Saxena & Basit, 1982).

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Secondary plant factors of Cucurbita species suppress sex attraction in the beetle Diabrotica undecimpunctata howardi

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Abstract

Males of spotted cucumber beetles, *Diabrotica undecimpunctata howardi* respond to a sex pheromone secreted by virgin females. The level of attraction of traps is influenced by the nature of the different host plants and their cucurbitacin levels: in fields of corn (*Zea mays*) without any known cucurbitacin content, males reach moderate population densities in late summer and early fall and are attracted to traps baited with female pheromone extracts. Simultaneously, much higher densities of beetles are visually recorded in fields of their alternate hosts, members of the family Cucurbitaceae. Although located in very close proximity to corn, pheromone traps in these fields were less effective in attracting beetles. Their ability to orient to pheromone traps seems to be negatively correlated with the cucurbitacin content of these crops. In behavioural assays plants containing cucurbitacin interfered with pheromone communication of this insect.

Key-words: sex attractants, *Diabrotica undecimpunctata howardi*, cucurbitacin, attraction, trapping

Behaviour modifying chemicals in insects regulate many of their important activities like sex attraction, mating, host finding, host acceptance, and feeding (Kogan, 1976). Examples of such chemicals in the vast literature of insect plant relationships are plentiful (Chapman & Bernays, 1978). Less obvious are the more subtle effects of secondary plant factors on sex pheromone communication systems of insects. Some examples of mating promotion were recently reviewed by Hendry (1976). In contrast, this report focusses on the suppressive influence of secondary plant compounds in Cucurbitaceae, on the sex pheromone communication system of the leaf beetle *Diabrotica undecimpunctata howardi* Barber.

Materials and methods

The beetles are reared in the laboratory from larvae feeding on corn roots, similarly to the procedures of Branson et al. (1975), but with modifications by Andersen (1981) and Kucera (1976). Emerging beetles were divided into sexes according to White (1977).
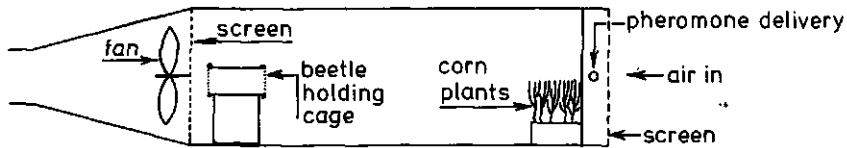
Fig. 1. Flight tunnel for assays of beetle orientation towards a pheromone source.

Males respond to a sex pheromone secreted by virgin females (Hummel & Kucera, 1977). Sex pheromones were collected on Tenax® absorbent from a purified (carbon filter) and rehumidified air stream of 500 ml/min passed over 50-100 at least two-day old virgin beetles in a glass tube. Pheromones were extracted from the absorbent and from the walls of the glass tube with a 1:1 n-pentane-diethylether mixture. They were concentrated at low temperature, tested, standardized and stored at $-20°C$ in closed vials.

For pheromone bioassays in the laboratory a cylindrical plexiglass flight tunnel was used (Figure 1). Pheromone concentrate was applied at the center line of the upwind end of the flight tunnel on metal discs similar to Gaston et al. (1971). Directed flight from the holding cage to the exit of the pheromone delivery system was evaluated. Young corn plants served as landing targets. For evaluating the inhibitory effects of cucurbits on sex pheromone induced flights, a standardized slice of cucurbit was placed into the holding cage together with the beetles. Under pheromone stimulation, beetle activities were recorded at one-minute intervals for 10 min. Male beetles for these bioassays were used only once per 24-hour period.

In the field the numbers of beetles in $1-m^2$ plots of *Zea mays, Cucurbita moschata* and the hybrid *C. andreanna* x *C. maxima* were assessed by three visual counts in nine replicates.

For pheromone bioassays in the field, standardized samples were volatilized from the center of sticky traps. Their height was adjusted to canopy level for cucurbits and to ear level for corn plants. The cucurbit varieties originated from the cultivar collection of A.M. Rhodes at Urbana, Illinois.

Results

Laboratory bioassays   Five minutes after stimulation with pheromone doses of 16 female beetle hour equivalents, an average of 50% of the beetles arrive in the upwind half of the tunnel (control without cucurbits, Figure 2). Pre-exposure of the beetles to the non-bitter variety *C. pepo* (zucchini) results in upwind flight orientation statistically indistinguishable from the control. In contrast, pre-exposure of beetles to bitter hybrids, e.g. *C. pepo* × *C. texana* (0.48 mg cucurbitacin/g of fresh weight) suppresses pheromone orientation to, and arrival at, the target almost completely, even at the highest possible doses. The beetles, however, notice the pheromone stimulus by transient antennal movements, walking, tarsal cleaning and decreased feeding intensity. Soon thereafter, compulsive feeding patterns described by Chambliss & Jones (1966) and Metcalf et al. (1980) are resumed.
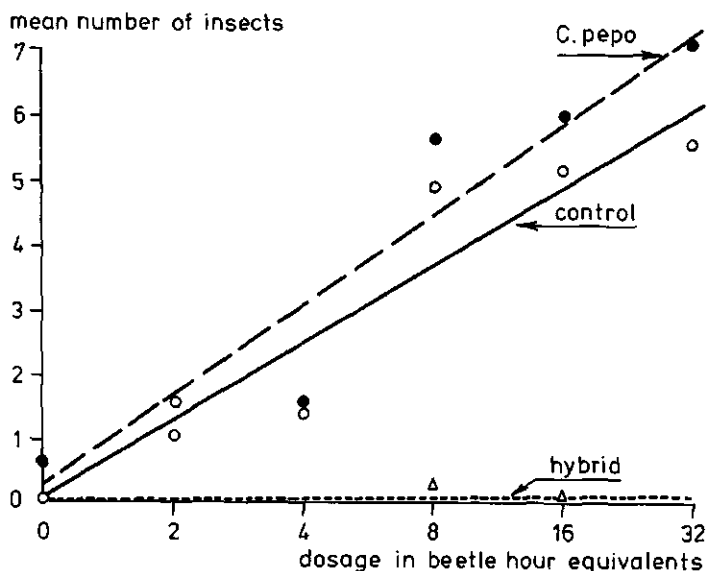
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Fig. 2. Upwind flight response of male *Diabrotica undecimpunctata howardi* to various dosages of a standardized sample of sex pheromone extract; number of insects in upwind half of flight tunnel. Tests were performed while the beetles were in contact with the fruit of *Cucurbita pepo* × *C. texana* hybrid (high cucurbitacin content), *C. pepo* (without cucurbitacin), and without any fruit (control). Three replicates were performed at each dosage level. The three lines are calculated according to the principle of the least squares of error.

Field bioassays   In the field, higher numbers of beetles were observed on cucurbits than in nearby *Z. mays*. On the bitter hybrid *C. andreana* × *C. maxima*, 20.74 ± 1.51 beetles, in the non-bitter *C. moschata* 4.15 ± 0.46 beetles, and in *Z. mays* only 1.33 ± 0.28 beetles were detected in areas of 1 m^2, respectively. In contrast, the mean net catch of pheromone traps in the respective fields is 0.3, 2.6, and 6.6 beetles, respectively (Table 1), showing higher catches in the corn field. In the crops *Z. mays* and *C. moschata* (both without cucurbitacines), no or little random flight occurred as indicated by the low number of beetles in the control traps. In the bitter hybrid, however, beetles are incapable of directed flight to the pheromone point source, as indicated by the small difference in total catch between control and 'treated' traps. In preliminary experiments performed under laboratory conditions, some isolated cucurbitacins (without plant volatiles) suppressed pheromone communication in the beetles. It can be concluded that cucurbitacins may play an important role in the observed suppression of the sex pheromone communication system.

Discussion

Cucurbitacins can be seen as behaviour modifying chemicals with a variety of different functions: Nielsen (1978) found cucurbitacins B, D, E and I in the crucifer *Iberis* sp. where they are potent feeding inhibitors of *Phyllotreta nemorum*, also a

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Table 1. Comparison of the number of *Diabrotica undecimpunctata howardi* beetles caught in pheromone baited traps in *Zea mays* and in cucurbits without and with cucurbitacins.

Host plant	Total number of beetles caught in pheromone traps[1]		Mean net catch per trap
	control	treatment	
Zea mays	0	93	6.6a[2]
C. moschata[3]	7	40	2.6b
C. andreana × *C. maxima*[4]	43	47	0.3b

1. 14 replicates for 1 h each with 500 beetle hour equivalents of pheromone.
2. a is significantly different from b at $P < 0.05$.
3. Cucurbitacin absent from foliage and fruit (Metcalf et al. 1980).
4. Cucurbitacin content 0.79 mg/g fresh foliage (Rhodes et al., 1980).

chrysomelid beetle. Chambliss & Jones (1966), Howe et al. (1976), Metcalf et al. (1980), and Rhodes et al. (1980) described the role of cucurbitacins as potent kairomones, arrestants, and feeding stimulants for the spotted cucumber beetle and related chrysomelids. In addition, our data support a hitherto undescribed suppressive function on mating communication of the spotted cucumber beetle while it is associated with its cucurbit hosts.

This study contributes to the poorly investigated field of mating pheromone suppression by host plant factors. To our knowledge, this is the first example in which such an effect has been supported by some behavioural experiments both in the laboratory and in the field. Van der Pers et al. (1980) provided some electrophysiological observations on interactions between plant odours and pheromone receptors in small ermine moths. Before, Hummel (1979) briefly reported the suppression of feeding and sex pheromone response of spotted cucumber beetles by *l*-bornyl acetate, a component of the volatile monoterpene fraction of camphorweed, *Heterotheca* sp.

In view of the rapid speciation and evolution of diabroticite beetles, intricate ecological relationships can be predicted at the interface between cucurbitacin containing plant systems and insect sex pheromone communication systems.

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Diel periodicity of take-off and immigration flights of the rice brown planthopper Nilaparvata lugens

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Abstract

Diel periodicity of take-off and immigration flights of *Nilaparvata lugens* in the Philippines was crepuscular. Take-off flights of macropters monitored in a rice field showing hopperburn symptoms were bimodal; immigration flights monitored in fields planted with a susceptible rice selection were unimodal, peaking around sunrise. Most immigration activity occurred during the vegetative stage of crop growth. Low light intensities, relatively lower temperatures and correspondingly higher relative humidities, and weak winds were recorded at take-off and immigration flight peaks.

Key-words: Nilaparvata lugens, immigration, flight, trapping

Take-off and immigration flights of the rice brown planthopper *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) are directly related to the insect's survival in a transient habitat such as a highly seasonal rice crop (Saxena et al., 1981). The largely monophagous habit of the insect (Saxena, unpublished) further limits its chances of survival in nature. *N. lugens* must escape from senescent and nutritionally depleted rice crops and colonize verdant rice fields to survive.

Flight is only by macropters, which are abundant toward crop maturity and in rice fields showing hopperburn symptoms (Saxena et al., 1981). Their ability to migrate and withstand a certain level of food deprivation (Kisimoto, 1977; Kusakabe & Hirao, 1976; Hirao, 1979a) increases their likelihood of arriving at distant breeding sites.

In tropical Asia, *N. lugens* only became a major rice pest during the last decade (Dyck & Thomas, 1979). Although the ecology of this pest, including flight periodicity, has been studied extensively in Japan, where it has been one of the most important pests of rice over a long period (Hirao, 1979b; Kisimoto, 1977; Kuno, 1977), marked climatological differences between tropical Asia and temperate Japan and inadequate information on macropter flight behaviour in the tropics necessitated a separate investigation. Periodicity of take-off and immigration flights of macropters was monitored along with meteorological factors that could influence flight behaviour. Because flight activity is involved in the major behaviour patterns

of *N. lugens*, this study may be useful in some theoretical and practical aspects of pest management.

Materials and methods

Take-off flight periodicity was determined by analyzing 24 hourly macropter catches made on 7 days from 13-19 October 1980, in a rice field showing hopper-burn symptoms in Laguna Province, Philippines. Upwind and downwind catches with 30 cm diameter, 1.5 m long sweepnets were made by sweeping about 1 mm above the rice foliage canopy. Each hourly catch was 20 sweeps 2 m from the edge of each of 5 sites showing hopperburn symptoms. The tip of the sweepnet was maintained during each sweep. Each hour's samples of macropters were placed in coded plastic bags for sorting, sexing, counting and recording in the laboratory.

Immigration flight periodicity was monitored December 1979 — March 1980 and May — August 1980 in rice fields planted with a susceptible rice selection IR1917-3-17 at IRRI, Los Baños, Philippines. Yellow pan traps and yellow board traps were used to capture *N. lugens* macropters. Yellow pan traps were plastic containers 17 cm in diameter and 21 cm high, each holding about 2 ml vegetable oil and 1 l water (Saxena, 1982). One week after rice was transplanted, 32 traps were staked at 2-metre intervals around the borders of each of 220-square metre plots. Height of the traps was maintained approximately 5 cm above the crop canopy. A plastic lid covered each trap from 18h00 to 05h00 and trapped macropters were counted hourly from 06h00 to 18h00 during the cropping season.

Yellow board traps 45 × 45 cm were coated on both sides with tanglefoot sticker. One week after rice was transplanted, 32 traps were staked facing the prevailing winds, four 1-metre-high and four 2-metre-high traps randomly distributed in each of four plots of 220 m². These traps were left uncovered at night. Trapped macropters were counted hourly from 06h00 to 18h00 during the cropping season.

In both studies, temperature and relative humidity were monitored at the rice foliage canopy level with a Serdex hygrothermograph, wind speed and direction with a Makino anemometer, and light intensity with a Li-cor photometer.

Results

Cumulative hourly catches of *N. lugens* macropters at take-off indicated distinctly crepuscular bimodal take-off activity, with the major occurring around dusk (Figure 1). Proportions of males and females were almost equal (1.1 ♂ : 1.0 ♀). The light intensity of < 10 lux, ambient temperature of 21-25°C, relative humidity of 80-100%, and weak winds of 0.3-1.2 km/h prevailing around dawn and dusk seemed to favour take-off (Figure 2). Patterns of downwind and upwind catches were identical. Sporadic nighttime catches may be attributed to the waxing moon phase which coincided with the observation period.

The maximum number of immigrant macropters recorded at 06h00 in both types of traps — pan traps which were closed overnight, and board traps, which were left uncovered at night in both cropping seasons indicated a distinctly *unimodal* immigration flight activity peaking around sunrise (Figure 3). The somewhat higher

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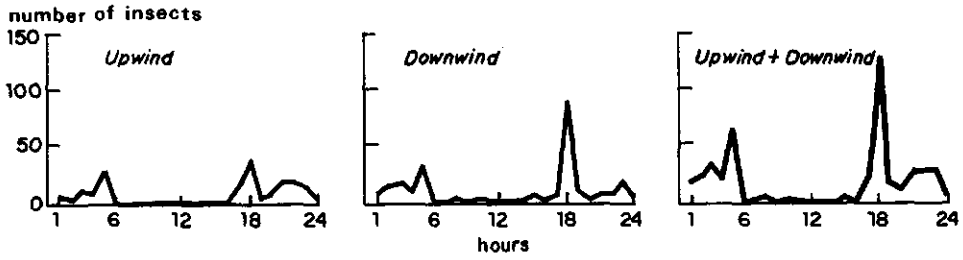
Fig. 1. Trends in hourly take-off on *N. lugens* macropters from a rice field showing hopper-burn symptoms. Hopper numbers are based on cumulative catches in 7 consecutive days, numbers in 100 sweeps per sampling site.
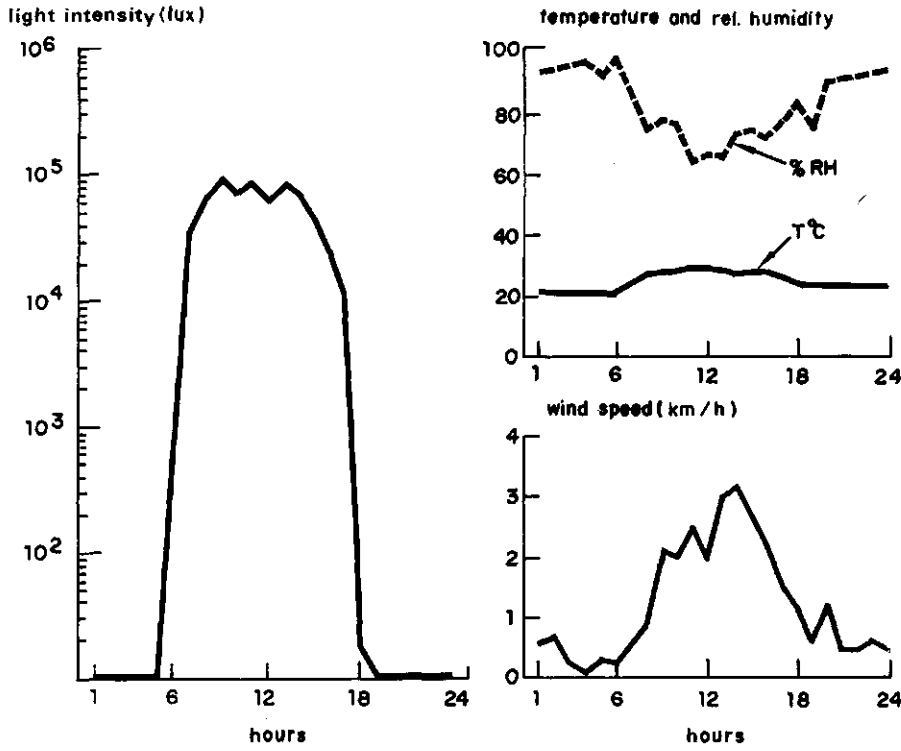
Fig. 2. Mean light intensity, temperature, relative humidity, and wind speed recorded at each hour of the day in the rice field where take-off flights of *N. lugens* macropters were monitored.

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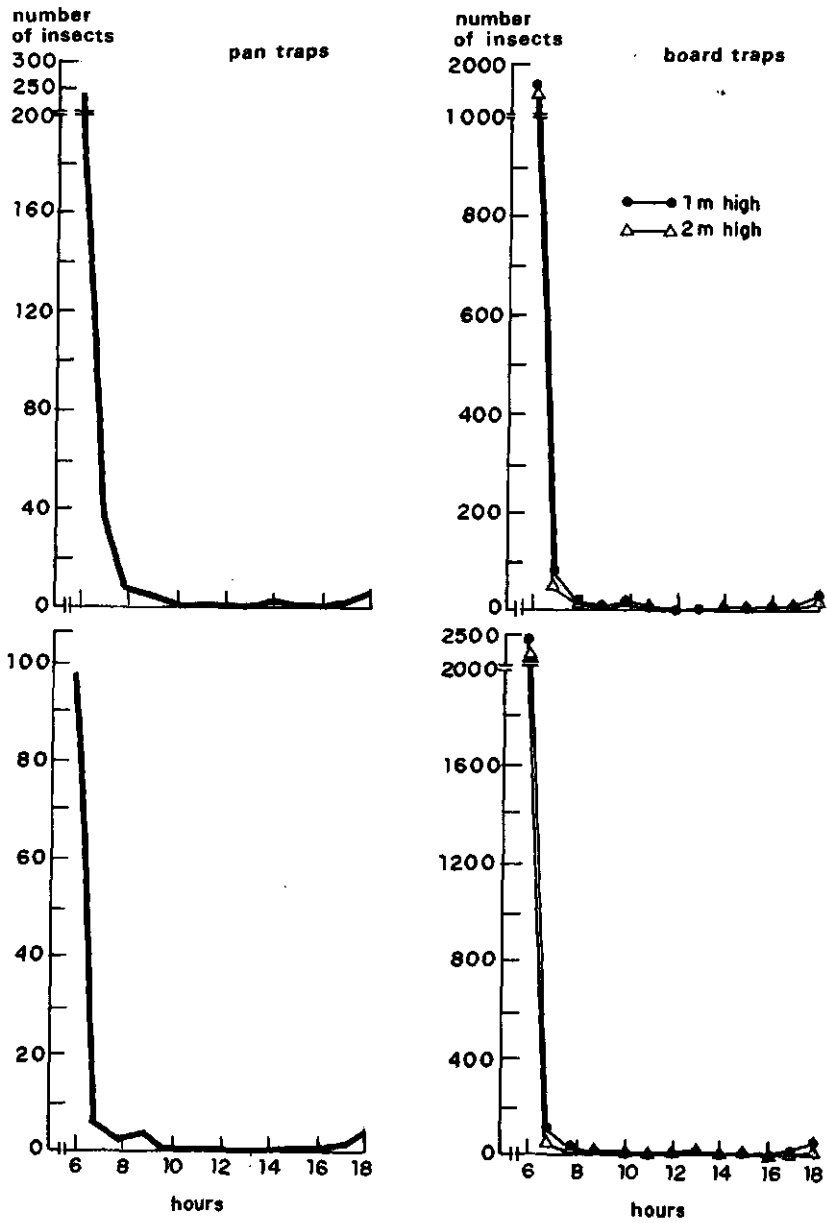
Fig. 3. Trends in daily catches of immigrant *N. lugens* macropters in rice fields planted with a susceptible rice selection. Hopper numbers are based on cumulative hourly catches in 128 yellow pan traps (left), and 16 yellow board traps (right), each set for 61 sampling days (December 1979-March 1980, top) and 63 sampling days (May-August 1980, bottom).
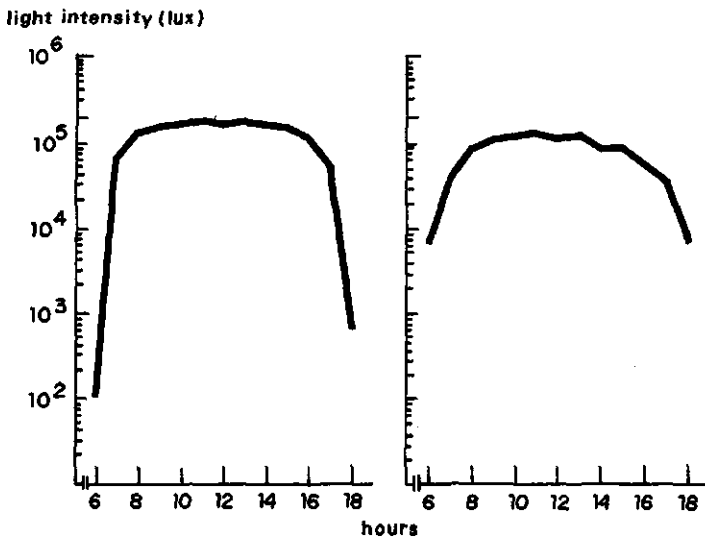
Fig. 4. Mean light intensity recorded at each hour of the day in rice fields in December 1979-March 1980 (left) and May-August 1980 (right).

catches in board traps than in pan traps can be attributed to the much larger trapping surface area and to the location facing the prevailing winds. Hourly catches of macropters in 1-metre-high and 2-metre-high board traps were almost identical, suggesting that trap height did not change trapping efficiency. Light intensity at 06h00 was about 100 lux in the first cropping season and 6 000 lux in the second (Figure 4). In general, winds were weak, temperature was relatively lower (20-23°C), and relative humidity was correspondingly higher (91-96°C) in rice fields when catches of *N. lugens* immigrants were recorded. Most immigration activity occurred during the vegetative stage of crop growth.

Discussion

A striking feature of the life cycle of *N. lugens* is its behavioural and physiological adaptation to the seasonality of the rice crop. When food resources become limiting in a crop nearing maturity or in rice fields showing hopperburn symptoms, more macropters are produced. They disperse and colonize new rice fields. But once macropters leave the humid microenvironment of their habitat, they face starvation and desiccation.

In spite of reported tolerance of *N. lugens* macropters to up to 7 days food deprivation, they succumb easily to desiccation (Hirao, 1979a). Since *N. lugens* is a small insect — macropterous ♂ 2.3-2.4 mm; macropterous ♀ 2.8-3.2 mm, excluding forewings (Okada, 1977) — with a correspondingly large evaporative surface area in proportion to mass, water conservation becomes a prime physiological problem during flight and dispersal. Baker et al. (1980) reported that macropters of tropical origin lost 28% of their initial body weight in a flight of about 8 h; after the first hour

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approximately 5% of the initial weight was assumed to be lost. Most of the weight loss was attributed to water loss, and it was inferred that environmental humidity was an important limiting factor in the insect's flight.

However, desiccation of airborne macropters can be attributed not only to environmental humidity, but also to the combined effect of light intensity, temperature, relative humidity, and wind speed. The interaction of these factors would determine the evaporation power of the air and influence desiccation rate of macropters in flight.

Diurnal changes in light intensity (illuminance) and sun ray direction are highly consistent, particularly in the tropics, and are much greater than similar changes in temperature, relative humidity, and wind speed (Figures 2 and 4). Thus, intensity and light direction may be the most reliable environmental cue (the 'Zeitgeber' of Aschoff, 1954) with which insects synchronize their daily biological activities of feeding, mating, oviposition, and flight. In-depth studies conducted by Fraenkel & Gunn (1940) and Wellington (1948) have shown that insect behaviour can be regulated by light conditions.

Diel periodicity of take-off and immigration flights of *N. lugens* macropters also seems to be strongly influenced by diurnal changes in light conditions. Around dawn and dusk, when peak catches of emigrant macropters are made, the contrast between sky and ground illumination is striking. At these times of the day, the sky is faintly illuminated, but the ground is nearly dark. The intensity of incident light ($<$ 10 lux) is so weak that the reflected light from the ground is inadequate for discriminatory vision in a rice field. This highly directional, unequal illumination apparently initiates the take-off of macropters, since other physical factors do not change drastically.

These light conditions, which persist relatively longer in the evening than in the morning, may account for the major peak of the crepuscular take-off activity of macropters observed at dusk. Inhibition of take-off activity at other times of day could be attributed to intense illumination. Sporadic catches at night also could be attributed to the diffused moonlight during the waxing moon phase which coincided with the observation period.

Peak light trap catches of *N. lugens* macropters in Malaysia were recorded in the first 2 hours after dusk (Lim, 1978). A major peak of flight activity occurred at around sunset in Guadalcanal (MacQuillan, 1975) and in the evening in the Philippines (Perfect & Cook, 1982). But the prevailing light conditions were not monitored quantitatively in these studies.

Ohkubo & Kisimoto (1971) reported that most flight activity at crop level in Japan occurred at dawn and at dusk, when light intensity adequate for flight activity was 1-200 lux. Maximum flight activity was at 100 lux.

Body temperature also seems to be a factor. Macropters in a rice field showing hopperburn symptoms were observed to prepare during the day for the peak exodus around dusk. At noon, the majority climbed to the upper surfaces or near the tips of leaves and oriented the long axes of their bodies parallel to the sun's rays. This postural control of solar input probably permits the body temperature to rise to the desired level, but prevents undue heating.

Such postural control of solar input to regulate body temperature has been re-

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ported for many insect species (May, 1979). According to Johnson (1966), the minimum temperature needed for take-off is usually 2-3°C higher than that needed to keep insects flying. The threshold of temperature for brown planthopper take-off flights in Japan was reported to be above 16.5°C; the morning take-off flight was suppressed when temperature fell below this level (Ohkubo, 1973). In the Philippines, take-off peaks were recorded when ambient temperatures were 21-25°C, relative humidity was 80-100%, and winds were weak, 1.2 km/h.

N. lugens macropters are weak fliers because of their light weight and relatively large wing expanse. Once they become airborne, they are likely to be carried by the wind, causing short- to long-range displacement, depending upon wind velocity (Rosenberg, 1981). In calm to weak winds, hopper dispersal may be somewhat local. Those that take-off at dawn are likely to be transported over greater distances, since wind speed is usually higher during the day than at evening or night.

Recently, it has been shown that the flight potential of *N. lugens* macropters varies from short to long durations (Padgham, D., personal communication). The dawn fliers probably represent those endowed with the greater fuel reserves more suited for longer flights. According to Baker et al. (1980), flights started in the morning lasted about four times longer on the average than those started in the afternoon. The longest tethered flight, about 11 h, was made by a field-collected female. According to Johnson (1976), it is a common observation in flight studies that most insects make short flights and only a small proportion fly for long periods. Shaw (1970) found that a population of alate aphids could be divided into migrants, fliers, and non-fliers.

The periodicity of *N. lugens* immigration flights is poorly understood. In most reports, flight activity was monitored either at a crop stage when take-off and immigration flights overlapped, or when devices that did not discriminate between emigrants and immigrants, such as light traps (Lim, 1978) and aerial suction traps (Perfect & Cook, 1982), were used. Hopper catches in yellow pans are better indicators of immigration flight activity. Kisimoto (1968) reported that in Japan, yellow pan traps attracted immigrant *N. lugens*, small hopper *Sogatella furcifera* (Horvath), particularly in the June and July immigration period. Saxena (1982) used a lightweight, yellow pan oil-water trap to monitor *N. lugens* colonization of rice fields in the Philippines.

We avoided any overlap between take-off and immigration flights by monitoring take-offs in a rice field showing hopperburn symptoms and immigration activity in a separate field in two cropping seasons. Macropters caught during the vegetative stage of crop growth represented only the immigrants.

Their strikingly unimodal activity peaked around sunrise, when light measured about 100 lux during the first cropping season (December 1979 — March 1980) and 6 000 lux during the second (May — August 1980). At these illumination levels, the yellow colour of the traps and the green foliage of the rice crop are distinctly visible, in sharp contrast to the colour of the sky. Almost similar alighting behaviour of the colonizing *N. lugens* macropters in yellow traps and on rice hills in a field (Saxena, 1982) indicates that their immigration activity in an area is primarily visually oriented. Saxena & Saxena (1975) reported that visual stimuli from plants, particularly colour, attracted a cotton leafhopper *Empoasca devastans* distant from up to 3.6 m

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away. Humidity and odour stimuli were effective only within 1 cm.

Differences in visual stimuli from rice plants and their background at different crop growth stages may also determine the amount of immigration in a rice field. Immigration activity is generally high during the midvegetative stage of crop growth, when the rice canopy is lush green. On the other hand, young seedlings in a freshly transplanted rice field with a water depth of 3-5 cm are not attractive to air-borne hoppers, although other physical and biological factors are favourable to immigration. The inadequate visual stimuli from young seedlings probably fail to elicit an optimum alighting response. The reflection of the sky on the field's water surface further reduces this response, much like the reported repellent effect of aluminium foil between plant rows to arriving alate aphid vectors of plant viruses (Smith et al., 1964; Kring, 1972).

Around sunrise, adequate perception of visual stimuli from yellow traps or from verdant rice fields seems to evoke a quick alighting response from aerial *N. lugens* macropters. That may account for the peak catches of immigrants around sunrise and the abrupt drop later in the day. Other physical factors, such as temperature, relative humidity and wind speed, also favour hopper landing at sunrise. The low activity of aerial predators at that time further increases the likelihood of successful arrival of the insect at new breeding sites. Moericke (1955) reported a sudden fall of low-flying aphids to considerable distances, by ceasing wing-beating or by flying downward in response to a yellow surface appearing beneath them in the field. However, according to Thomas et al. (1977), the flying aphids' natural mode of descent when the air was calm was by gentle downward flights, but the insects occasionally resorted to sudden falls to considerable distances in a state of reflex immobilization.

The marked diel periodicity of both take-off and immigration flights of macropters has an adaptive significance in the life of *N. lugens*, a defenseless and weak flier vulnerable to desiccation. The crepuscular flight behaviour may serve as a means of offsetting the adverse effects of environmental inclemencies and of avoiding visually orienting predators.

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Ecology

Generalist versus specialist utilization of host plants among butterflies

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Abstract

For three oligophagous butterflies, viz. the European swallowtail *Papilio machaon*, the wood white *Leptidea sinapis*, and the orange tip *Anthocharis cardamines*, data have been assessed on: (1) the availability of different potential host plants at three localities, (2) larval survival (under laboratory conditions) on the different plants available, and (3) the actual field distribution of offspring on the different plants. The results show that the butterflies exhibit different degrees of food specialization as follows: (1) *P. machaon* utilizes only 1 plant of the 9 potential host plants available; (2) *L. sinapis* utilizes 6 out of 11 plants available, and (3) *A. cardamines* utilizes 15 out of 18 plants available. General theory predicts that specialization should be found in species faced with constant or abundant resources, whereas generalization should be favoured when resources are inconstant, unreliable, or rare. The relevance of this theory as an explanation for the different degrees of food specialization found among the three butterflies is analyzed with respect to (1) between year variation in availability of host plants, (2) between year variation in offspring survival on different plants, and (3) relative abundance of host plants.

Key-words: evolution, *Papilio machaon*, *Leptidea sinapis*, *Anthocharis cardamines*, generalists, specialists, food preferences, oviposition

Herbivorous insects may generally be divided into three groups with regard to the degree of feeding specialization, viz. monophagous, oligophagous and polyphagous species. Polyphagous insects are characterized by their feeding on a number of taxonomically distantly related plants. Among butterflies the polyphagous 'lifestyle' is exemplified by, for instance, *Polygonia calbum*, the larvae of which can be found on plants belonging to at least 8 different families (Seppänen, 1970; pers. obs.). Oligophagous insects are generally defined as feeding on a number of plants, all of which however, must belong to the same family. In fact, as shown by Ehrlich & Raven (1964), the majority of butterflies seem to be oligophagous. Monophagous insects, finally, feed on one species of host plant only. However, different kinds of monophagy can readily be recognized, for example one might involve geographic restriction as a result of only one plant species available within the geographical range inhabited by a butterfly population. This kind of monophagy has been called 'ecological monophagy' by Gilbert (1979). A typical example of this kind of mono-

phagy is furnished by *Gonopteryx rhamni* which virtually only feeds on *Rhamnus frangula* in Scandinavia but incorporates additional *Rhamnus* species in its diet at more southern latitudes as the number of *Rhamnus* species that are available increases. Another kind of monophagy is exemplified by insects which use only one species as host plant although closely related, congeneric, plants are readily available. This kind of monophagy corresponds to the 'coevolved monophagy' of Gilbert (1979). An example is furnished by *Colias palaeno* which is monophagous on *Vaccinium uliginosum* throughout the Holarctic although three additional congeneric plants are generally available, at least in the Scandinavian habitats (Petersen, 1954).

When aiming to investigate the host plant utilization strategies of butterflies, and especially when trying to evaluate the importance and the effect of different selective pressures influencing the evolution of these strategies, both monophagous and polyphagous species may be rather difficult objects of study. The reason why monophagous species are rather unsuitable is that they have no freedom of choice on account of their having become so specialized that the larvae can only survive on one species of host plant. This confers that monophagous insects cannot respond to selective pressures by broadening, or narrowing, their food niche. Polyphagous species may be unsuitable as well since they use many different, distantly related, host plants in their breeding habitats. This may imply that the choice of host plant is not a very critical factor in the life of many polyphagous butterflies, and may imply that dimensions other than that of the food niche are of primary importance for the establishment and survival of these butterflies. However, oligophagous species are very useful study objects for two reasons:
– They can use different host plants only so long as these belong to the host plant family; a fact which indicates that the choice of host plants is a critical issue.
– Their food niche always exhibits a certain width conferring that it is possible to evaluate the effects of various selective pressures as the insects respond to these by either narrowing or widening their food niche.

In this paper I show that three oligophagous butterflies viz. the European swallowtail, *Papilio machaon*, the wood white, *Leptidea sinapis*, and the orange tip, *Anthocharis cardamines*, exhibit different degrees of host plant specialization and discuss the role of:
– between-year variation in availability of different host plants
– between-year variation in offspring survival on different plants
– the abundance of host plants, as organizing forces for the different degrees of host plant specialization exhibited by the three butterflies

In part this paper is a synthesis of earlier published work on the host plant biology of the three butterflies (Wiklund, 1974, 1975, 1977; Wiklund & Åhrberg, 1978), but several new field data and unpublished experimental results will be presented here for the first time. Furthermore, I will briefly present some results of a 5-year field study on the distribution of eggs, and offspring survival, on 18 different crucifers available to a central Swedish population of *A. cardamines*. The details, and complete results of this study will be published elsewhere.

Host plant utilization of Papilio machaon

P. machaon is oligophagous on various plants in the Umbelliferae (Apiaceae). The number of umbellifers available to a south Swedish population of *P. machaon* at Vejbystrand was assessed by surveying a 3-kilometre coastal stretch regularly inhabited by the butterfly. The distribution of first instar larvae on the umbellifers was estimated by surveying all plants, with exception of *Aegopodium podagraria* and *Anthriscus silvestris* of which a sample of one thousand plants each was surveyed, every third day between 20 June and 8 July 1981. The potential host plant range of the larvae from this population has earlier been determined by rearing larvae on all plant species and observing the mortality on each species (Wiklund, 1975).

The results show that 9 umbellifers out of the 12 species present in the habitat were included in the potential host plant range of *P. machaon* (Figure 1). However out of these 9 species only one, *Angelica archangelica*, was actually used as host plant (Figure 1). Thus, although larvae of *P. machaon* have been found on 19 different umbellifers in Fennoscandia (Wiklund, 1974), the butterfly population studied at Vejbystrand was effectively monophagous. This specialist utilization of host plants seems to be typical of most Fennoscandian populations of *P. machaon*, although the actual umbellifer species specialized on may differ between localities (Wiklund, 1974; in prep.).

The host plants that are oviposited on by *P. machaon* are typically very conspicuous, or 'apparent' sensu Feeny (1976), and females can be seen approaching suitable plants from a distance of some 10 m. The females appear to have a 'search image' for leaf shape, just like *Battus philenor* (Rauscher, 1978), and can sometimes be seen approaching non-host plants that bear a superficial resemblance to the 'correct' host plants (Wiklund, 1974b). However, the females have never been seen to actually alight on a non-host plant during the oviposition flight, and thus seem able to discriminate between suitable and unsuitable plants by airborne stimuli alone. I suggest that a prerequisite for the use of this kind of time-saving search behaviour is that the host plants are highly apparent, i.e. stand out from the rest of the vegetation so that their quality can be adequately assessed from a distance.

Other butterflies that use highly apparent host plants and never seem to alight on non-host plants during the oviposition flight include *Gonopteryx rhamni* the females of which locate twigs of *Rhamnus frangula* among branches of *Betula* and *Salix* species with never-failing accuracy, and females of *Palaeochrysophanus hippothoe* which alight on the elongate inflorescenses of *Rumer acetosa* and oviposit in crevices on the stem as they climb down the stem of the inflorescence.

Host plant utilization of Leptidea sinapis

L. sinapis is oligophagous on plants in the Leguminosae (Fabaceae). The density of the various leguminous plants available to a central Swedish population of *L. sinapis* at Ingarö was assessed by counting plants in 0.3 × 0.3 m quadrats randomly thrown in the locality inhabited by the butterfly. The frequency with which the butterflies used different leguminous species as host plants was assessed by following individual females and noting on which plants the eggs were deposited (Wiklund,
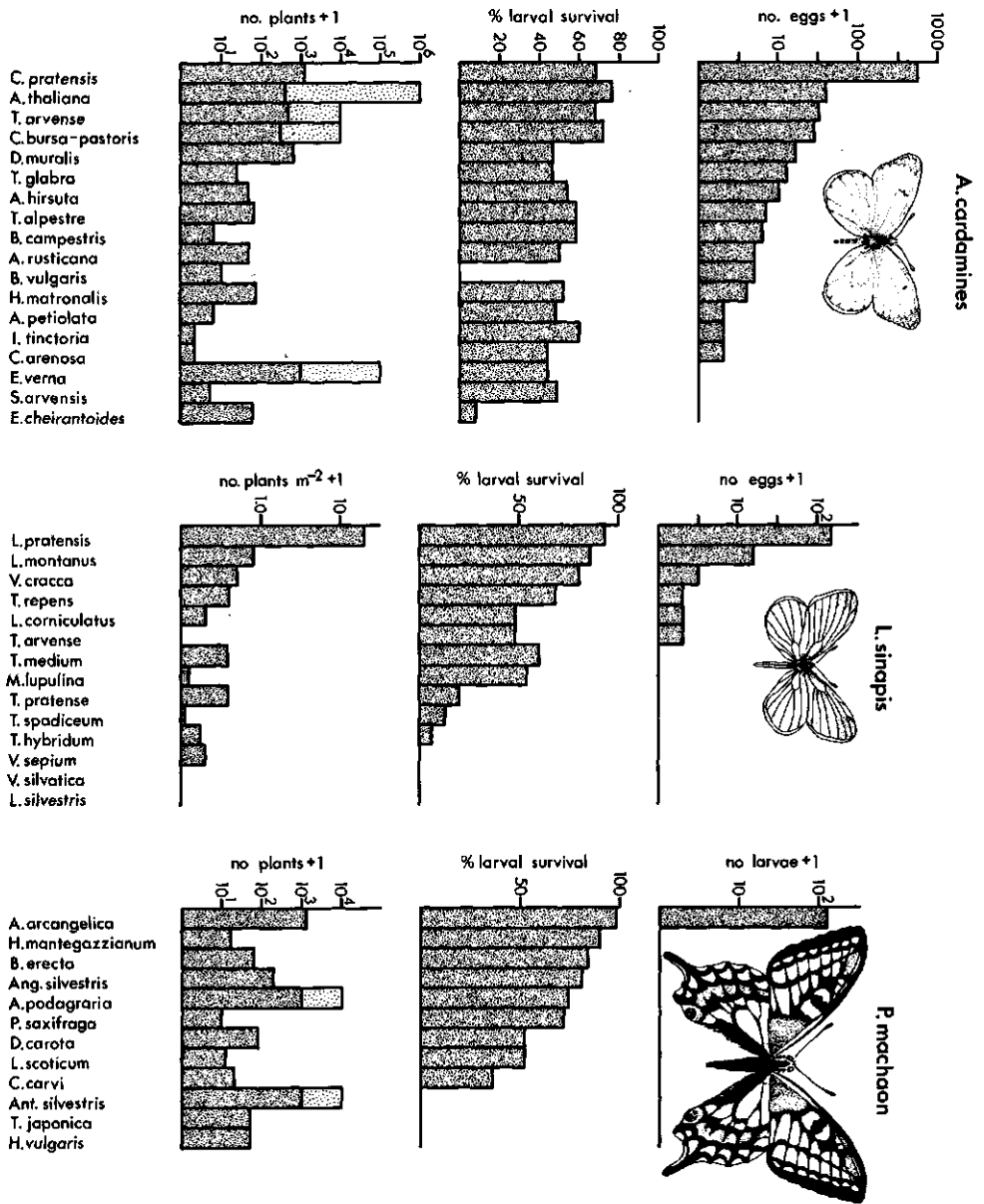
Fig. 1. The bottom row pictures the number of potential host plants available to *Papilio machaon* and *Anthocharis cardamines* at two localities (the number of plants that were surveyed for eggs of larvae is indicated by the dark part of the histograms). For *Leptidea sinapis* the density of potential host plants was assessed at a third locality. The middle row shows the larval survival on the different plants under laboratory conditions, and the top row pictures the actual distribution of offspring on the plants at the three localities. Since all scales are log scales + 1 is added to all values.

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Table 1. Plants alighted on by a female *Leptidea sinapis* between 11h14 and 13h54 on 5 June on Ingarö during which time 19 eggs were laid, viz. 17 on *Lathyrus pratensis*, 1 on *Lathyrus montanus* and 1 on *Vicia cracca*.

Plant species	Number of alightings
Graminae sp.	58
Lathyrus pratensis	17
Achillea millefolium	15
Galium mollugo	10
Anemone nemorosa	7
Vicia cracca	6
Trifolium repens	6
Trifolium medium	6
Galium verum	5
Lathyrus montanus	4
Vaccinum myrtillus	4
Taraxacum officinale	4
Trifolium pratense	3
Galium boreale	3
Alchemilla vulgaris	3
Melampyrum silvaticum	3
Veronica chamaedrys	3
Filipendula ulmaria	3
19 additional spp.	24
Total	184

1977). The potential host plant range of *L. sinapis* was determined by placing 15 newly hatched larvae on all leguminous plants and observing the mortality on each plant species.

The results show that 11 out of the 14 leguminous plants present in the habitat were included in the host plant range of *L. sinapis* (Figure 1). However, out of these 11 species only 6 were used as host plants. Furthermore, 87% of the eggs were laid on *Lathyrus pratensis* and 10% on *Lathyrus montanus*, resulting in 97% of the eggs being laid on only 2 out of the 11 potential host plants. Thus, *L. sinapis* at Ingarö does exhibit a specialist utilization of the potential host plants available although, admittedly, not as specialized a utilization as that exhibited by *P. machaon* at Vejbystrand.

The searching behaviour of *L. sinapis* during the oviposition flight is completely different from that of *P. machaon*. The fundamental difference is that *L. sinapis* seems unable to distinguish between unsuitable and suitable plants without alighting on them. When searching for plants to oviposit on, female *L. sinapis* only fly short distances at a time, frequently alighting on small plants in the herbage layer. Although the most favoured host plant *L. pratensis* is highly abundant in the habitat it is alighted on with a frequency of only some 9% (Table 1). A possible explanation for this fact is that the *L. pratensis* specimens are only a few cm high during the oviposition period of *L. sinapis* and are thus highly 'unapparent' among the grasses

and other herbs that grow in the dense low herbage layer.

Other butterflies thas use 'unapparent' host plants i.e., low ground-dwelling herbs that do not stand out from the surrounding vegetation, include *Fabriciana adippe* and *Lycaena phlaeas* both of which can be seen frequently alighting on non-host plants during the oviposition flight. Thus, the inability of these butterflies to recognize a suitable host plant in flight may be due to the unapparency of their host plants.

Host plant utilization of Anthocharis cardamines

A. cardamines is oligophagous on various plants in the Cruciferae (Brassicaceae). The number of different crucifers available to a central Swedish population of *A. cardamines* at Ljusterö has been assessed by counting the number of flowering specimens twice a week, from the middle of May until the middle of June, during a five-year period between 1976 and 1980. The distribution of eggs on the crucifers, and larval survival, has been assessed by inspecting all crucifers twice a week between the middle of May and the end of July during the same five-year period. All plants bearing eggs or larvae were marked with a numbered piece of coloured tape. If the abundance of a particular crucifer greatly exceeded a thousand specimens, only a sample of plants was studied. The potential host plant range of *A. cardamines* was determined by placing between 15 to 25 newly hatched larvae on all crucifers and observing the mortality on each plant species.

The results show that 17 out of the 18 crucifers present in the habitat in 1976 were included in the host plant range of *A. cardamines* (Figure 1). When reared on *Barbarea vulgaris* from Ljusterö larval mortality is 100%. However, when reared on *B. vulgaris* from certain other areas larval survival is comparable to that on other crucifers. In 1976 eggs were found on 15 of the 18 available crucifers (Figure 1). When taking into account that the phenology of the three remaining crucifers is such that they escape in time from the ovipositing females, it seems apparent that *A. cardamines* exhibits a generalist utilization strategy in relation to the crucifers at Ljusterö.

The searching behaviour of *A. cardamines* during the oviposition flight is quite similar to that of *P. machaon* in so far as females are never observed to alight on non-host plants. *A. cardamines* exclusively oviposits on crucifers when in flower and females in flight seem to use the bright colour of the petals as visual cues of identification from a distance of 5-10 m. However, female *A. cardamines* often refuse to lay eggs on crucifers that already bear conspecific eggs (Wiklund & Åhrberg, 1978). This egg load assessment (Rothschild & Schoonhoven, 1977) takes place after the female has alighted on a crucifer, indicating that females of *A. cardamines* are highly discriminative both during the pre- and post-alighting phase of oviposition, just like *Euphydryas editha* (Rauscher et al., 1981).

Differences between specialist and generalist strategies

Although specialization and generalization are relative concepts and only have meaning on a comparative basis, it is often possible to assign a given butterfly spe-
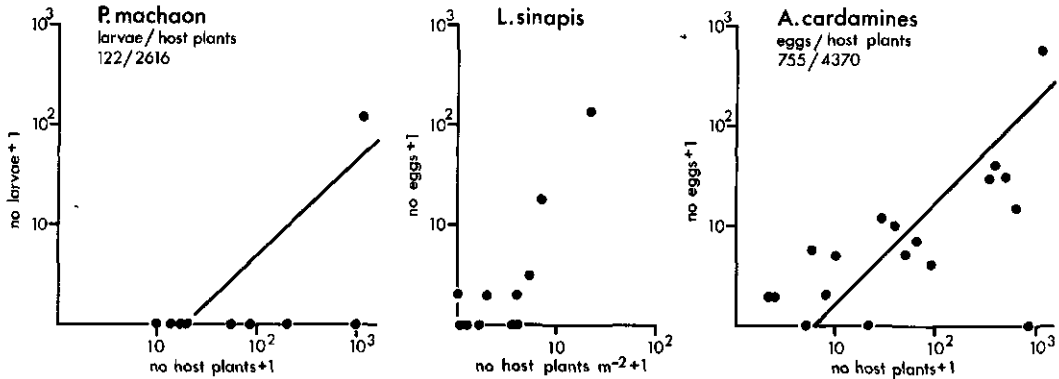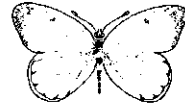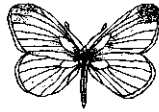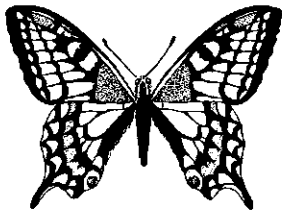
Fig. 2. The relationship between host plant abundance and the distribution of offspring on different plants. For *Papilio machaon* and *Anthocharis cardamines* the line indicates the ratio between number of offspring and plant abundance that would be expected if host plant utilization was directly proportional to plant abundance. Since all scales are log scales + 1 is added to all values.

cies to one category or the other. One profound difference between a specialist versus a generalist strategy is reflected in the relationship between the abundance of potential host plants and the frequency with which they are oviposited upon.

From an adaptationist standpoint a specialized usage of host plants should be expected when one plant consistently gives a higher number of surviving offspring than other plants. As long as this most suitable host plant is abundantly available all other potential host plants should be avoided regardless of their abundance. As can be seen in Figure 2 the larvae of *P. machaon* were found only on *A. archangelica* although *A. podagraria*, on which larval survival is high under laboratory circumstances, is completely avoided in spite of its being 10 times more abundant. The mechanism with which *P. machaon* implements this specialist strategy seems to involve profound differences in oviposition preferences between plants (Wiklund, 1981).

Conversely, a generalized utilization of host plants should be favoured when differences in the suitability of different plants are small. Thus all potential host plants available should ideally be oviposited upon. To achieve this end differences in female oviposition preferences between potential host plants should be small, conferring that host plants should be oviposited on approximately in proportion to their abundance. However, if one samples specimens in a plant population by picking a random point and finding the closest plant, one will have an over-representation of

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isolated plants (Pielou, 1969). Since this procedure probably is a good approximation of the host plant searching behaviour of butterfly females, one should expect generalist strategists to oviposit on isolated plants more often than would be expected from their abundance, and conversely, densely aggregated plants to be oviposited on less often. In fact, this relationship seems to hold for *A. cardamines* at Ljusterö (Figure 2).

From a specialist/generalist viewpoint *L. sinapis* seems intermediate between *P. machaon* and *A. cardamines*. *L. sinapis* exhibits a high preference for the two most abundant leguminous species, *L. pratensis* and *L. montanus*, other plants e.g., *T. repens*, *T. medium* and *T. pratense*, are avoided and oviposited upon less often than would be expected from their abundance (Figure 2).

Ecology and evolution of specialization and generalization

What selective pressures bring about the evolution, or the maintenance, of specialization and generalization? Basically it is often assumed that specialists exploit their particular environment better than do closely related generalists (Slobodkin & Sanders, 1969), and it has also been suggested that the greater efficiency and the consequent division of labour is the 'ultimate reason we have so many species' (MacArthur, 1972). Even though recent work by Scriber & Feeny (1979) and Futuyma & Wasserman (1981) seems to refute the hypothesis that specialized insects exhibit greater physiological feeding efficiency than do generalists, Fox & Morrow (1981) in a recent review conclude that 'local feeding specialization can be produced by biochemical, behavioural, ecological, and evolutionary processes'. Thus, feeding specialization among insects seems to be fairly well understood, and the fact that most phytophagous species have a more restricted diet in the field than they could have (as indicated by rearing experiments; cf. Dethier ref. in Levins & MacArthur, 1969), specialization seems to be almost ubiquitous among insects.

In comparison, feeding generalization among insects seems both less common and less well understood. However, general theory predicts that whereas specialization in resource use is likely to be favoured in species faced with relatively constant or abundant resources, generalization should be favoured when resources are inconstant or unreliable or when they are rare (Futuyma, 1979). To discuss the relevance of this theory in relation to butterfly host plant utilization I will attempt to discuss the host plant biology of *P. machaon*, *L. sinapis*, and *A. cardamines* in relation to:
— between year variation in abundance of different host plants
— between year variation in offspring survival on different plants
— relative abundance of host plants

Variation in host plant availability   The basic hypothesis is thus that differences in suitability exist between host plants. In consequence, the host plants can be arranged in a hierarchy according to their relative suitability. In a stable, or predictable, environment these differences should in the long run lead to the utilization of the most suitable host plant only. However, in an unstable, or unpredictable, environment the availability of a given host plant may be highly uncertain in different

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Table 2. Predictability of potential host plants of *Papilio machaon* at Vejbystrand (Umbelliferae), *Leptidea sinapis* at Ingarö (Leguminosae) and *Anthrocharis cardamines* at Ljusterö (Cruciferae) as judged by the ratio annual-biennial versus perennial plants.

Plant family	Number of plant species	
	annual-biennial	perennial
Umbelliferae	2	7
Leguminosae	3	8
Cruciferae	.12	6

years. Thus, even if one given host plant is most suitable, the unpredictability of the opportunity to use this species regularly, should lead to the utilization of other host plants as well.

In a tentative way the predictability of plants can be deduced from whether they are annual, biennial or perennial. In short, perennial plants are expected to be more predictable than annual or biennial plants. Since the majority of the umbelliferous and leguminous plants used by *P. machaon* and *L. sinapis*, respectively, are perennial, whereas the majority of the crucifers used by *A. cardamines* are annual or biennial, the general theory seems corroborated (Table 2).

The high between year variation in plant abundance is also shown by the five year study at Ljusterö (Wiklund, in prep.). To quote some examples: the abundance of *A. thaliana* decreased from 10^6 to 501 between 1976 and 1980; the abundance of *C. pratensis* decreased from 1384 to 141 between 1977 and 1979; the abundance of *T. glabra* increased from 28 to 802 between 1976 and 1978.

Variation in offspring survival Another way in which between year variability could affect the host plant utilization of butterflies involves the relative survival of offspring on different plants in different years. Again, if offspring survival is consistently highest on one plant, specialization on that plant should be favoured. However, if the relative survival of offspring on different plants varies between years, and especially if the variation on some plants is negatively correlated with that on others, a generalist strategy should be favoured. This would be likely to apply if the host plants used by a particular butterfly grow in different habitats and offspring survival is dependent on weather conditions which cannot be predicted at the time when the actual host plant choice is made i.e., at oviposition.

Among other plants *A. cardamines* females regularly oviposit on *Arabidopsis thaliana*. This plant usually grows on dry hillocks with a soil layer not exceeding a few cm. Another important host plant is *Cardamine pratensis* which grows in very wet meadows or in dug ditches. During hot and dry summers larval mortality is high on *A. thaliana* on the hillocks since the plants often die of desiccation before

the larvae have had time to complete their development. In contrast, larvae on *C. pratensis* do not suffer any mortality due to hot and dry weather conditions. On the other hand weather-induced larval mortality is high on *C. pratensis* during rainy summers when large numbers of larvae may die from drowning. In contrast, larvae on *A. thaliana* seem to suffer little increased mortality on account of the weather during rainy summers. This negative correlation with respect to offspring survival on *A. thaliana* and *C. pratensis* is clearly indicated by the five year study at Ljusterö (Wiklund, in prep.). Furthermore, the unpredictability of offspring survival on different plants is indicated by the fact that only *Capsella bursa-pastoris* regularly produced surviving offspring during the five years of study at Ljusterö although no less than 9 crucifers were oviposited on all of the years (Wiklund, in prep.).

Unfortunately nothing is known about between year variation in offspring survival on different plants used by *L. sinapis*, whereas the utilization of one host plant only by *P. machaon* of course precludes even the possibility of between plant variation.

Relative abundance of host plants   In general abundant availability of host plants should be expected to allow, and favour, the evolution of a specialist strategy. Conversely, low abundance of several host plants should 'necessitate' the adoption of a generalist strategy. Essentially the relevant parameter is not abundance per se, but rather a combination of plant abundance and apparency which together influence the probability that a given plant will be alighted on by an ovipositing female.

It is striking that *P. machaon* oviposits on a highly abundant and apparent host plant, and also exhibits an extreme specialist strategy (Figure 2). For *L. sinapis* the use of an extreme specialist strategy may be impossible due to the low visual apparency of the host plants (Figure 2). The number of offspring in relation to the number of host plants surveyed is highest for *A. cardamines* (Figure 2). During the five year study at Ljusterö this ratio varied between 12% and 22%. Since *A. cardamines* also exhibits egg load assessment and largely refrains from ovipositing on crucifers that already bear eggs, the high relative use of available crucifers implies that females may have difficulties in finding suitable host plants. This inference is also supported by the fact that ovipositing females at Ljusterö have been observed flying for 1-2 h without encountering a single crucifer. In consequence, the low relative availability of suitable crucifers may be a factor which favours a generalist strategy of host plant utilization in *A. cardamines*.

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Resource exploitation by gall wasps of the genus Diplolepis

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Abstract

Three species of *Diplolepis* wasps simultaneously induce distinct galls on the immature leaflets of *Rosa* species. Structural differences in these galls illustrate how gall insects have managed to manipulate plant growth to their own advantage. Gall insects do not partition merely the resources of host plants, but rather they exploit latent growth and developmental potentialities of their host tissues.

Key-words: Diplolepis species, Cynipidae, galls, *Rosa* species

Amongst the many specialized guilds of phytophagous insects, perhaps the most intricate is that in which certain insects compel their hosts into producing a gall. Galls are atypical plant growths that appear in response to a stimulus applied by the immature stages of select insects. Most gall insects are host, organ and tissue specific and they take advantage of a fundamental property of plants whereby definitive tissues can be converted to an immature state. Once the tissues are in this state, the insect is able to direct morphogenesis to form the gall. Only the insect gains in the association; the host plant, via the gall, provides the insect with highly nutritious food and a sheltered chamber while the plant experiences structural and physiological damage.

Galls differ widely in size, shape, and structure with each species of insect inducing characteristic galls (Mani, 1974; Rohfritsch & Shorthouse, 1982). Amongst the most structurally complex galls are those induced by wasps of the family Cynipidae. The purpose of this paper is to review some of the intricacies of the gall-inducing guild by examining the galls of three closely related cynipids of the genus *Diplolepis*.

Biology of Diplolepis

Diplolepis wasps are small insects (adults average 7.0 mm in length), all of which induce galls on *Rosa* spp. There are about 30 Nearctic species of *Diplolepis* (Krombein et al., 1979). All are organ specific and attack either the flowers, leaves, buds, stems, or roots of their hosts. The three species of *Diplolepis* discussed in this paper

i.e., *D. polita* Ashmead, *D. lens* Weld and *D. nebulosa* Bassett, are mainly found on *Rosa acicularis* Lindl., but they can be found also on *R. woodsii* Lindl. and *R. blanda* Ait. (Shorthouse, 1975).

The life cycles of all *Diplolepis* are similar (Shorthouse, 1973, 1975). They are univoltine and overwinter inside their galls in the prepupal stage. The pupal stage lasts about 15 days and occurs in the early spring once diapause has broken. The adults emerge inside their galls, chew to the outside, and search for tissues suitable for oviposition. This period of emergence is synchronized with the stage of host development that is optimum for gall initiation. *Diplolepis* adults live for only 3-4 days outside their galls; however, most populations have an extended period of emergence enabling them to overlap the period of tissue susceptibility.

Eggs are laid with one end of the egg slightly embedded into immature tissues (Shorthouse, 1974, 1975). The egg stage lasts from 7-15 days and the galls are initiated once the larvae begin to feed. The galls grow rapidly and the larvae, that feed on the highly nutritious cells of the gall, are fully grown in about 2 months.

Morphology of cynipid-induced galls

Galls induced by cynipids are characterized by a definitive size and shape. They are composed of tissues differentiated into well defined zones, that are concentrically arranged around the larval chamber. The organization and contents of cells composing these galls are substantially different from those of unaffected host organs (Maresquelle & Meyer, 1965; Rohfritsch & Shorthouse, 1982). Specialized cells called nutritive cells line the chamber and are the only source of food for the insect. The nutritive layer is often 8-10 cells thick and is composed of two types of cells. Cells in direct contact with the insect are cytoplasmically dense and contain an enlarged nucleus. They are uniformly rich in acid phosphatases, soluble sugars, and amino-soluble products (Bronner, 1977). Adjacent cells are vacuolate and often contain starch granules. Nutritive cells continue to proliferate as long as the insect is feeding (Rohfritsch & Shorthouse, 1982).

Beyond the nutritive layer is a zone of hard layer tissue composed of thick-walled lignified cells. These sclerenchyma cells, also referred to as mechanical tissue, were once thought to serve a protective function, but most authors now agree that their role is one of support (Fourcroy & Braun, 1967). Vascular tissue is found between the nutritive and hard layers and is connected to the vascular bundles of the host organ. Beyond the hard layer is an extensive layer of cortical parenchyma cells. These are usually the largest cells of the gall and are thought to act as water storage tissue (Maresquelle & Meyer, 1965). The organization and functioning of gall tissues are discussed in further detail by Rohfritsch & Shorthouse (1982).

Morphology of Diplolepis-induced leaf galls

At least 8 of the North American species of *Diplolepis* induce galls on leaflet tissues. Galls of the three species discussed here are from Saskatoon, Saskatchewan, and Sudbury, Ontario. Eggs of the three species are laid at approximately the same time in the early spring (Shorthouse, 1975) while the leaflets are still enclosed in
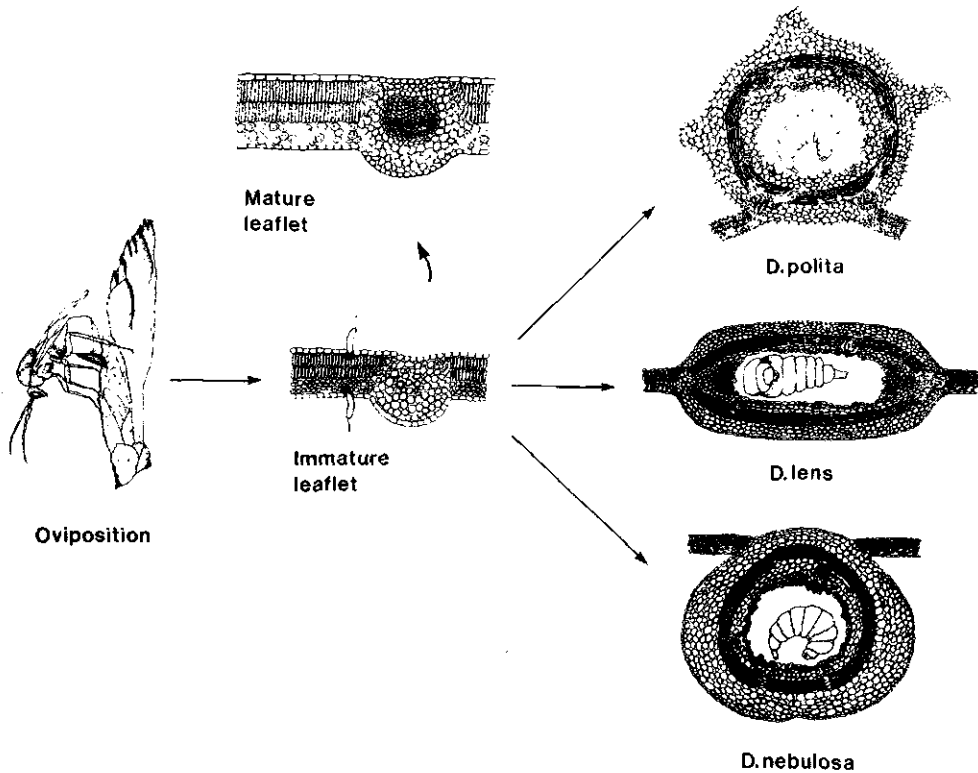
Fig. 1. Schematic presentation of basic events in the formation of galls induced by cynipid wasps of the genus *Diplolepis*. A *Diplolepis* ovipositing into a flushing leaf bud of *Rosa acicularis* is shown to the left. The leaflet sections (centre) show the arrangement of host cells at the time of oviposition (note the egg of *D. polita* on the adaxial surface and the egg of either *D. lens* or *D. nebulosa* on the abaxial surface) and cellular arrangement of unaffected tissue at maturity (above). The size of each gall (on right) is shown in relationship to the size of leaflet tissues to which it is attached. Nutritive cells line the larval chambers. The dark zone which circumscribes each gall is the hard layer composed of lignified sclerenchyma cells.

flushing buds (Figure 1). Eggs of *D. polita* are laid on the adaxial surface, whereas those of *D. lens* and *D. nebulosa* are laid on the abaxial surface. Palisade and spongy mesophyll cells are differentiated at the time of oviposition; however, they are smaller and more closely packed compared to those of mature leaflets (Figure 1).

Freshly hatched larvae begin feeding on either palisade or sprongy mesophyll cells even before they are free of the egg shell. Cells nearest those fed upon by the larva enlarge and proliferate and within a few days the larva is enclosed in its chamber, surrounded by several layers of nutritive cells.

Mature galls of *D. polita* are spherical, 3.5 to 5.0 mm in diameter, clothed with weak spines, and occur in clusters on the adaxial surface of leaflets (Figure 1). The nutritive layer is one or two cells thick, whereas the adjacent layer of vacuolate

starch-laden cells is about 5 cells thick. The hard layer matures to a thickness of about 10 rectangular cells and circumscribes the larval chamber. Lignification occurs evenly in all hard layer cells until the lumen of each is occluded and the cells appear as a solid mass. A zone of cortical parenchyma cells, 3-4 cells thick is found beyond the hard layer zone. Greatly enlarged epidermal cells cover the entire gall surface (Figure 1).

Mature galls of *D. lens* are lenticular-shaped, 4.5 to 6.0 mm in diameter, and protrude from both adaxial and abaxial leaflet surfaces (Figure 1). A thick zone of nutritive tissue composed of irregularly shaped cells encircles the larval chamber in the lateral regions. Few nutritive cells are found on the adaxial and abaxial chamber surfaces. The hard layer consists of two disc-shaped zones of rectangular sclerenchyma cells, one below and the other above the larval chamber (Figure 1). The lumen of cells in both layers is seldom more than half occluded. Epidermal cells are intact although stretched laterally.

Mature galls of *D. nebulosa* are spherical, 5.0 to 7.0 mm in diameter spineless, and occur in clusters on the abaxial leaflet surface (Figure 1). Nutritive cells surround the chamber throughout gall growth; however, two disc-shaped layers of sclerenchyma cells, one below and the other above the larval chamber, are differentiated while the gall is immature. Lignification of these cells occurs soon after they are differentiated and their lumen becomes about half occluded. These sclerenchyma discs do not expand as the gall matures but become separated by a zone of vacuolate nutritive cells. However, once the gall matures a hard layer composed of thick-walled lignified cells forms between the two discs thus encircling the chamber (Figure 1). The lumen of these cells becomes filled with phenolic-like substances in contrast to the disc-sclerenchyma cells which are free of the substances. A thick layer of cortical parenchyma cells occurs outside the circumscribing hard layer. The external layer of cells is composed of cortical rather than epidermal cells because the epidermal layer ruptures as the immature gall grows abaxially.

Discussion

Leaf galls induced by *Diplolepis* well illustrate the extent to which gall insects manipulate plant tissues. Each of the three galls described here is a harmoniously organized entity with a determinate growth resulting in an orderly arrangement of cell layers. They are structurally distinct even though they are initiated simultaneously from identical leaf tissues. Although each species induces similar nutritive cells, the hard layer differs. In galls of *D. polita*, for example, the first sclerenchyma cells are differentiated as the galls are maturing, whereas in galls of *D. lens* and *D. nebulosa*, disc-shaped layers of sclerenchyma cells appear soon after initiation. The lumen of hard layer cells in *D. polita* galls is occluded while those in *D. nebulosa* become filled with phenolic-like substances.

How gall insects are able to induce the proliferation of gall cells and control their contents is not understood. Perhaps the distinctive characteristics exhibited by galls of *Diplolepis* are due to slight variations in the way each species either wounds or stimulates plant cells through the actions of various secretions. Rohfritsch & Shorthouse (1982) suggest that gall insects solicit genetic expressions from their host cells.

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Whatever the mechanism, it is clear that the relationship gall insects have with their hosts is one whereby the insect rather than the plant organizes tissue development. Galls also show us how easily plant cells submit to reorganization when a new organizer is introduced.

The development of a gall involves processes that are normal to plant growth. Furthermore, the cells and tissues are abnormal only insofar as they appear in abnormal abundance and in unusual places. What is commonloy referred to as 'new' in gall formation is only a new combination of properties capable of being exhibited by the plant under specific conditions. In this respect, galls induced by *Diplolepis* are simply spheres of normal *Rosa* tissues which have been rearranged under a new morphogenetic coordinating influence. Even further manipulation can occur as a result of attack by inquilines of the genus *Periclistus* since the tissues remain pliable following initiation by the gall former (Shorthouse, 1975, 1980).

The documentation of the feeding habits of gall insects provides an opportunity to examine the niche width and overlap of closely related groups of animals. *Diplolepis*, for example, do not merely partition the resources of host plants as part of their niche, but rather they exploit latent growth and developmental potentialities of the tissues of those plants. Obviously further studies of gall development and experimental transfers of gall insects and their extracts are needed before our knowledge of these associations can be furthered.

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Inducible defences of white birch to a geometrid defoliator, Epirrita autumnata

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Abstract

Foliage of the white birch has two types of inducible responses to mechanical damage of leaves: a rapidly enhanced and rapidly relaxed resistance in leaves of the current year, and high resistance in the years after defoliation. The former tends to stabilize defoliator populations; the latter leads to destabilization. The rapidly inducible defensive response is almost equally efficient in damaged leaves and in intact adjacent leaves. This response is most efficient in birches from benign environments. The higher resistance in the years after defoliation is strongest during the first two years but significantly reduced the growth of *Epirrita* larvae even four years after defoliation. This response is strongest in birch from potential outbreak areas of defoliators. It can curtail the reproductive potential of *Epirrita* by more than half.

Key-words: induced plant resistance, *Epirrita autumnata*, *Betula pubescens*, outbreaks

Chemical factors creating resistance to herbivores in host plants may be continuously occurring or inducible. Inducible resistance reaches a high level after contacts with herbivores, usually after grazing. The existence of inducible forms of resistance can be explained by the fact that the high metabolic costs of resistance make a continuously high level of defence metabolically uneconomical.

The resistance of the white birch, *Betula pubescens*, to the geometrid defoliator *Epirrita* (= *Oporinia*) *autumnata* is largely inducible (Haukioja, 1980) and can be essential in the periodic fluctuation of population density experienced by *Epirrita* populations in northern Europe (Tenow, 1972).

There are two separate types of inducible resistance in birch foliage. One takes place in foliage that has suffered chewing or mechanical damage in general. It is triggered within (hours or) days and also relaxes within a few weeks. The other type appears in the leaf generations which the birch produces in the years following defoliation. These two types of inducible defences with different relaxation times have opposite effects on the stability of pest populations. Rapidly inducible defences with a short relaxation time are basically stabilizing. Their efficiency is strongest when the population density of the pest is high. The enhanced level of resistance in the years following defoliation is a potentially destabilizing agent. During the first year

after defoliation, the density of the pest population is probably still high but resistance may remain at a high level years after the collapse of the defoliator population and thus potentially delay the start of a new outbreak.

In this paper I present data which show the characteristic features of inducible defences with different relaxation times. Local and geographical variability in both types of defences is also discussed. The bioassays performed were intended to show the existence, not the maximal effects, of inducible defences in birch foliage.

Materials and methods

The experiments described below were performed at the Kevo Subarctic Research Station (69°45′N, 27°E) in northern Finland in 1979. The triggering of defensive responses by leaf damage was tested by means of bioassays where growth of *Epirrita* larvae served as an index of the suitability of foliage samples. Larvae were reared as described in Haukioja & Niemelä (1977) except that larvae in different treatments were siblings. Each experiment started with newly-hatched larvae.

The existence of a rapid increase in the defensive level of foliage was tested by rearing experimental caterpillars on damaged foliage from six birches growing on the lower slope of Jesnalvaara mountain (for details see Haukioja et al., 1978) and by feeding control larvae with undamaged foliage from adjacent birch trees. Experimental trees were damaged at least every second day by tearing pieces of leaf lamina. Leaves for control animals were picked each day from a new tree. Larvae were reared individually in plastic tubes at outdoor temperatures and were weighed to an accuracy of 1 mg 19 days after the beginning of the experiment.

The number of years during which the higher level of resistance persists after defoliation was tested by comparing the pupal weights of larvae reared on leaves from previously undefoliated control trees with those reared on leaves from trees which were defoliated two (in July 1977), three (in July 1976) or four (in July 1975) years earlier. Defoliation was manual. Leaves from trees defoliated in 1975 and 1976 were used the following year to raise larvae but the trees were not defoliated these years. The experiments started with 33 first-instar larvae per rearing vessel. Pupal weights were taken to an accuracy of 1 mg.

Results

Existence of rapidly inducible defence with short relaxation time Mechanical damage to leaves triggers the rapidly inducible defence in birch foliage during the growth period of leaves, in June — mid-July in Lapland (Haukioja & Niemelä, 1979).

Larvae reared on birch leaves from trees with torn foliage grew less rapidly than larvae reared on leaves from undamaged control trees (Table 1). This was true both when larvae were fed on leaves with scars of previous damage or on intact, adjacent leaves; this indicates that the induced change in foliage quality was not strictly local. The growth of larvae on leaves induced by previous damage was retarded more than in earlier experiments (Haukioja & Niemelä, 1977, 1979) for two reasons. Firstly, weights referred to larvae which had not yet completed the larval stage. To

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Table 1. Mean weight of 19-day-old larvae of *Epirrita autumnata* in relation to previous damage of leaves on which they were fed.

Treatment	Weight[1] (mg)					
	males			females		
	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n
Control (no damage even of adjacent leaves)	58.5a	13.07	41	55.1a	13.59	51
Leaves with scars of previous damage	35.9b	6.68	42	40.0b	8.54	48
Intact leaves adjacent to damaged ones	39.0b	9.05	45	41.8b	10.76	49

1. Differences between treatments were significant (ANOVA, $F = 84.5$, d.f. $= 2, 270$, $P _ 0.0001$) but not between sexes (ANOVA, $F = 0.7$, d.f. $= 1, 270$, $P = 0.39$). Means with same letters do not differ significantly (Student-Newman-Keuls test, $P - 0.05$).

some extent they can compensate final weights on poor diets by longer larval periods. Secondly, foliage damage which induced the response was made repeatedly in 1979 while it was made only once in earlier years. Consequently, stronger responses seem natural.

The moisture and nitrogen content of damaged foliage did not differ from values in control foliage whereas the phenolic content of leaves as well as the ability of foliage extract to inhibit trypsin was higher in leaves induced by damage (Niemelä et al., 1979).

There is both local and geographic variation in the responses of birches to previous damage. The above test was made with birches from the lower slope of the Jesnalvaara mountain where the existence of the response was also documented earlier. Trees on the more barren upper slope of the mountain exhibited either no response at all or only a weak response (Haukioja, 1980). A birch provenance from southern Finland had a stronger response than birches originating from the present study site (Haukioja, 1980). The available evidence therefore indicates that in *Betula pubescens* the rapidly inducible increase in defensive level is characteristic of birches in benign environments.

Existence of high resistance in years after defoliation Defoliations by *Epirrita autumnata* have not taken place in years with high summer temperatures (Niemelä, 1980). This may reflect the efficiency of predators and parasites in warm summers but also the efficiency of rapidly inducible defensive systems in such years. The fact that temperature is a very important limiting factor for trees in northern Finland is well documented (e.g. Sarvas, 1972). At poor growth sites and in cool summers regulatory factors cannot prevent *Epirrita* defoliations all years. The survival of birches in those areas reflects their ability to recover by seed production or/and by regrowth. The first year after defoliation obviously is critical in vegetative recovery because pest populations still must have a high density. But this is also the year when the resistance of birch foliage is high (Haukioja & Niemelä, 1977; Haukioja, 1980). In experiments approaching natural conditions (larvae were reared in mesh

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Table 2. Mean pupal weight of *Epirrita autumnata* in relation to defoliation history of birches on whose leaves they were reared.

Treatment		Weight[1] (mg)					
		males			females		
		$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n
Control (no previous defoliation)		73.2a	5.10	33	76.9a	8.47	36
Years since an artificial defoliation:	2	59.6b	5.38	20	58.8b	9.31	28
	3	70.0c	8.60	25	69.3	12.54	27
	4	68.0c	8.80	26	66.6c	9.79	37

1. Differences between treatments were significant (ANOVA, $F = 30.7$, d.f. $= 3, 224$, $P < 0.0001$) but not between sexes (ANOVA, $F = 1.2$, d.f. $= 1, 224$, $P = 0.33$). Means with same letters do not differ signifcantly (Student-Newman-Keuls test, $P > 0.05$).

enclosures on branches of trees with and without previous defoliation) the reproductive capacity of *Epirrita* suffered by more than half on trees which had been defoliated in the previous year (Haukioja, 1980, unpubl.).

Table 2 shows the results of an experiment performed to find out how many years resistance persists at a high level after defoliation. The success of larvae was poor two years after defoliation and the reduction in pupal weight was significant although milder even three and four years later. Table 2 cannot be used to evaluate the magnitude of the response in a truly natural situation because detached leaves are more usable than growing ones (Haukioja, 1980) and because defoliation alone is a weaker cue for high defence than defolation and *Epirrita* faeces in the soil (Haukioja, in prep.).

The content of foliage phenolics was significantly higher in birches defoliated in the previous years than in control trees. The trend was reversed in nitrogen content (Haukioja, unpubl.).

While rapidly induced defence with a short relaxation time was more efficient in birches from benign environments, the resistance in the years following defoliation was the opposite. Birches on the upper slopes of Jesnalvaara responded more efficiently to defoliation the following year than trees at lower altitudes (Haukioja, unpubl.). A provenance from southern Finland outside the outbreak range of *Epirrita* did not respond at all (Haukioja, 1980). Experiments in progress have confirmed that five provenances of *Betula pubescens* from potential outbreak areas did respond in the year after defoliation and that two provenances outside the outbreak range had an insignificant response (Haukioja & Kapiainen, unpubl.).

Discussion

Inducible resistance with a short and long relaxation time must be based at least partially on separate mechanisms because in some birches only one of them functions. The physiological basis of these systems is not known.

The defensive nature of the above responses may result from four types of causes.

They may be merely by-products of metabolic recovery processes of the foliage, they may result from general stress reactions in the tree, they may be based on non-specific defensive responses towards pathogens and herbivores, and they may be specific defensive responses to *Epirrita* and other important defoliators of the white birch. To distinguish between all these alternatives is not possible at the moment. Moreover, none of them excludes *Epirrita* defoliations as a causal factor affecting the evolution of responses adopted by birches.

The rapidly induced defence with a short relaxation period may reduce the probability of outbreaks of defoliators, except by reducing the reproductive potential of the pest population, because it allows time for predators and parasites to check the larval populations of the pest. This is in agreement with the more efficient reaction in birches at good growth sites for such areas tend to have a more diverse herbivore fauna offering a richer number of alternative hosts for generalized predators and parasites.

The high level of resistance during the recovery phase of birches is obviously the form of defence which best explains the ability of birches to live in spite of periodical peak densities of *Epirrita*. The same explanation applies to the tortricid moth, *Zeiraphera diniana*, defoliating *Larix decidua* (Benz, 1974; Fischlin & Baltensweiler, 1979).

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The phytophagous insect community and its impact on early successional habitats

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Abstract

Although the effects of insect herbivores on single plant species have received some attention, especially in the field of biological control of weeds, relatively few studies have addressed this question to natural plant communities. In this paper the relationship between plants and insects during the early part of a secondary successional gradient is described in terms of recently harrowed land in southern Britain which has been allowed to recolonise for different periods. The composition of the phytophagous insect community is analysed and the effects of these insects on the vegetation is determined in two ways. Firstly, by the experimental exclusion of insects from comparable sites and secondly, by the assessment of damage to selected plant species. From these long-term field studies the impact of insect herbivores on plant composition, structure and reproduction can be ascertained.

Key-words: successional habitats, insect diversity, plant diversity, *Trifolium repens, Raphanus raphanistrum, Spergula arvensis*, population dynamics

Herbivory plays a key role in the structure of plant communities in addition to being a selective force in the evolution of secondary chemicals and morphological features of plants (Feeny, 1970). Studies on the effects of herbivores on vegetation are often restricted to vertebrates (Duffey et al., 1974; Patton & Frame, 1981; Watt, 1981a,b); where the secondary consequences of grazing, mechanical damage and the deposition of dung and urine, often make the impact even more complex. There have been relatively few studies on insect herbivores and their effects on plant populations, and more particularly natural plant communities (Harper, 1977; Whittaker, 1979). The reason for this is likely to be that natural plant communities are rarely seen to suffer from insect damage (Hairston et al., 1960). Only in unnatural situations, such as crops, can their effects be manifest.

Insect herbivores have commonly been exploited in the biological control of weeds. Undoubtedly some of the best accounts of the role of insects in the structuring of plant populations come from this source (Harris et al., 1978; Harris, 1980), although even here attention is often focused on the insect. In other studies the effects of grazing have been restricted to single herbivore species and single plant species or species pairs as in the elegant work of Bentley et al. (1980). In other cases

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grazing has been studied under controlled conditions (Bentley & Whittaker, 1979) or simulated by the experimental removal of leaves (Lee & Bazzaz, 1980). Furthermore the plant species are commonly perennials and the insects monophagous. What effects do insect herbivores have on natural communities of annual plants where a reasonable proportion of the species are polyphagous? Such situations are typical of early successional habitats.

The aim of this paper is to investigate the effects of insect herbivores on the development and structure of natural plant communities characteristic of an early secondary successional habitat. The experimental system, at Silwood Park, Berkshire, involves four sites of different successional age. The sites were started from recently harrowed ground in March of each year and have been allowed to recolonise. Thus sites in the first, second, third and fifth years of succession were available for study. The vegetation is typical of that of disturbed land on sandy soil in Southern Britain.

The phytophagous insect community

Insect and plant species diversity are closely correlated during the first few years of succession (Southwood et al., 1979). It might therefore be predicted that the insects impact would be very considerable at this time. Indeed it is said that stable environments are less likely to show damage (Harper, 1977).

The number and type of insect herbivores must greatly influence their impact on the vegetation. Consequently, all above ground phytophagous species, which could be collected without destruction of the habitat, were sampled four times each season. Only those species in which the larvae feed internally in the plant tissue were excluded; the leaf miners being the subject of a separate study. Details of the sampling methods are given in Southwood et al. (1979). The number of species increases rapidly during the first two years of succession but slows subsequently (Figure 1). Although a reasonable proportion of the species are phytophagous (Brown & Southwood, in prep.) many are specialised to feed on a particular 'type' of plant. The insect species characteristic of this early stage of succession are generally short lived and closely synchronised with the phenology of the plant. There is, therefore, a rapid turnover of species during this time (Brown & Southwood, in prep.). Thus the composition of the insect fauna changes considerably during early succession and closely mimics the availability of the plant types.

Phytophagous insect species, excluding the mining and gall forming guild, may be classified into defoliators and non-defoliators or suckers. By removing leaves or sometimes shoots defoliating insects (mainly Curculionoidea and Chrysomeloidea, in this case) inflict some measurable damage of the vegetation, whilst sucking insects have a far more subtle effect on the plant. Consequently there have been very few studies of the effects of non-defoliating insects of plants (Dixon, 1971a, b). In the sites described here there are far more sucking insect species than defoliators, although the defoliators present are usually found in reasonable numbers as the relatively low levels of diversity (Williams α) indicate (Figure 2). But as Harper (1977) has stressed relatively low densities of insects can have a significant effect on a plant population providing the searching efficiency and dispersal rate of the insects are high. Such features are most certainly true of the early successional defoliators in
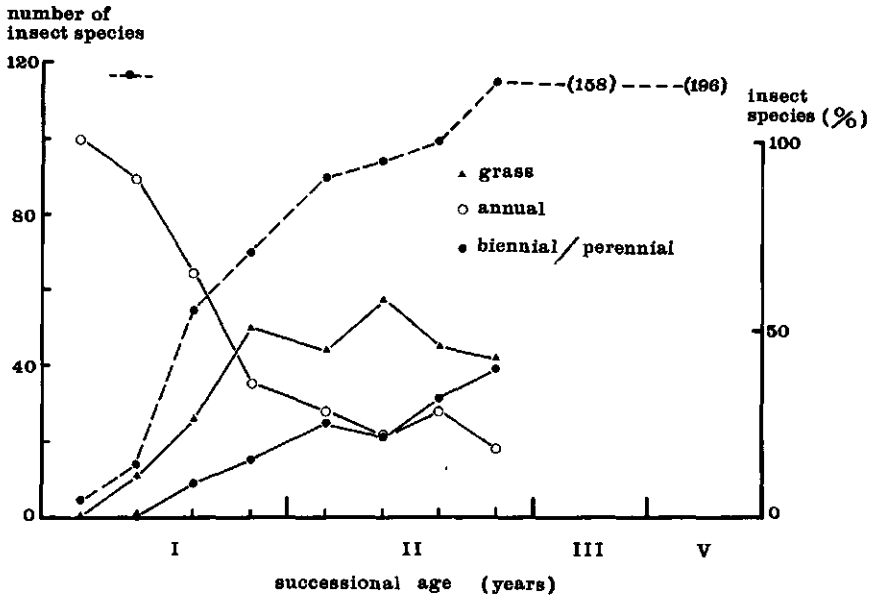
number of
insect species

120

(158) - - - - (196)

insect
species (%)

100

▲ grass
○ annual
● biennial / perennial

80

50

40

0

I   II   III   V

successional age (years)

0

Fig. 1. Accumulation of insect species during five years of succession, and the proportion of insect species feeding on the major plant types.
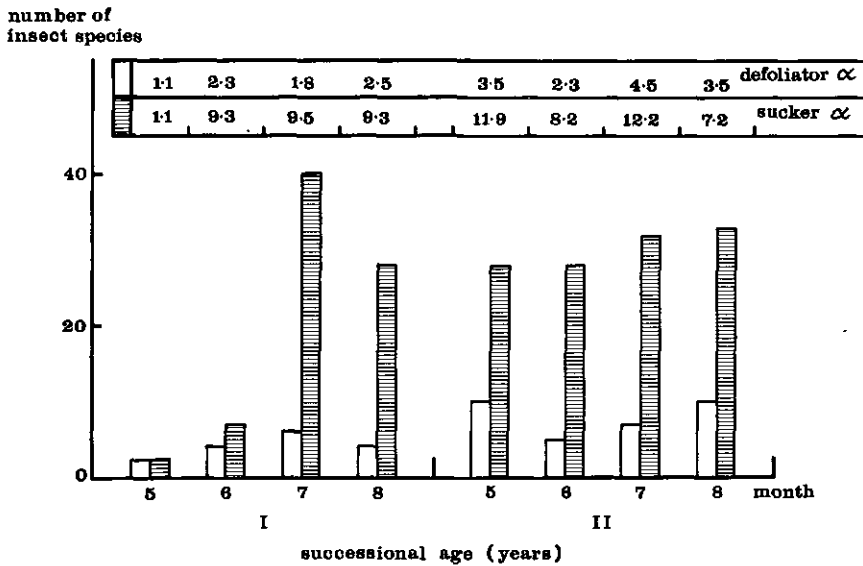
number of
insect species

| 1·1 | 2·3 | 1·8 | 2·5 | 3·5 | 2·3 | 4·5 | 3·5 | defoliator α |
| 1·1 | 9·3 | 9·5 | 9·3 | 11·9 | 8·2 | 12·2 | 7·2 | sucker α |

40

20

0

5   6   7   8   5   6   7   8   month

I   II

successional age (years)

Fig. 2. Seasonal variation in insect species richness and diversity of defoliating and sucking insects (Williams α) during the first two years of succession.

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this study. The sucking insects reach a peak in numbers in July of the first year of succession, but maintain a high species richness and diversity throughout the second year. The earliest colonisers are Heteroptera and Aphididae feeding on ruderal plant species, later the Cicadellidae are found as the grass species·invade (Figure 1).

Herbivore impact

Since the species diversity of phytophagous insects rises during the early years of succession, an increase in their impact might be expected. However, this is not the case (at least in the successional gradient under study). The insects associated with six dominant plant species in the first year *Spergula arvensis, Bilderdykia convolvulus, Plantago lanceolata, Stellaria media, Trifolium repens* and *Poa annua*; and in the second-fifth years *Cirsium arvense, Rumex crispus, Senecio jacobaea, Trifolium repens, Vicia sativa, Holcus lanatus*, have been sampled over the first five years of secondary succession. While the insect species diversity increases with the successional age of the habitat (Figure 3a) the impact on the plant species (measured as the percentage of the shoot biomass lost) shows a negative relationship, suggesting that the impact of insect herbivores is greater when their diversity is low, during the early stages of succession (Figure 3b). The first year site only shared one of the dominant plant species (*Trifolium repens*) with the other sites and cannot therefore be included in this relationship.

However, the impact of insect herbivores on *Trifolium repens* can be compared. This has been assessed in three ways — the number of leaves damaged — the leaf area removed and — the proportion of the biomass lost (Figure 4). Although the number of leaves attacked was relatively small in the first year of succession, the
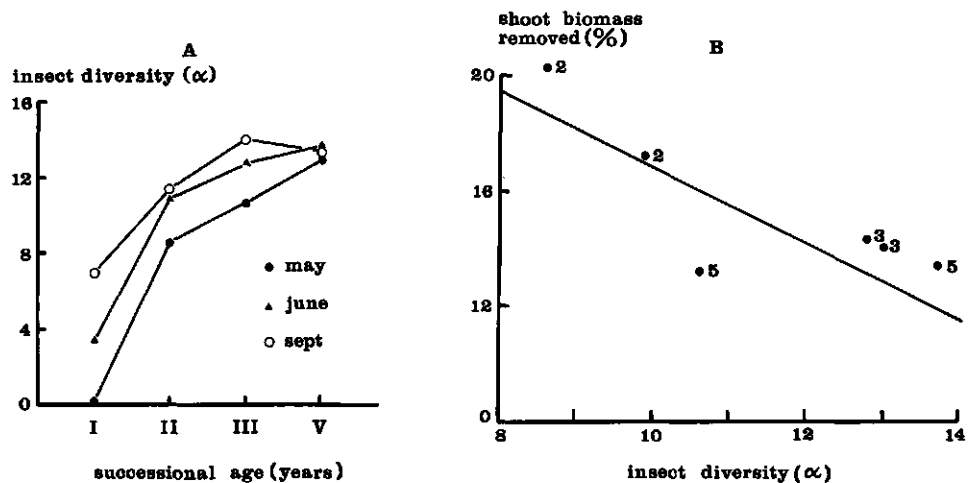

Fig. 3. A: Diversity of phytophagous insects (Williams α) associated with six dominant plant species. B: Relationship between insect diversity and the proportion of shoot biomass removed for six plant species on two sampling occasions. Figures on graph indicate the successional age of the site (in years). $r = -0.92$, significant at $P < 0.01$ (after Armes, unpublished).

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leaves damaged (%)
100
•—leaf area removed(%)
o—biomass removed (%)
40
60
20
20
0
I   II   III   V   successional age (years)
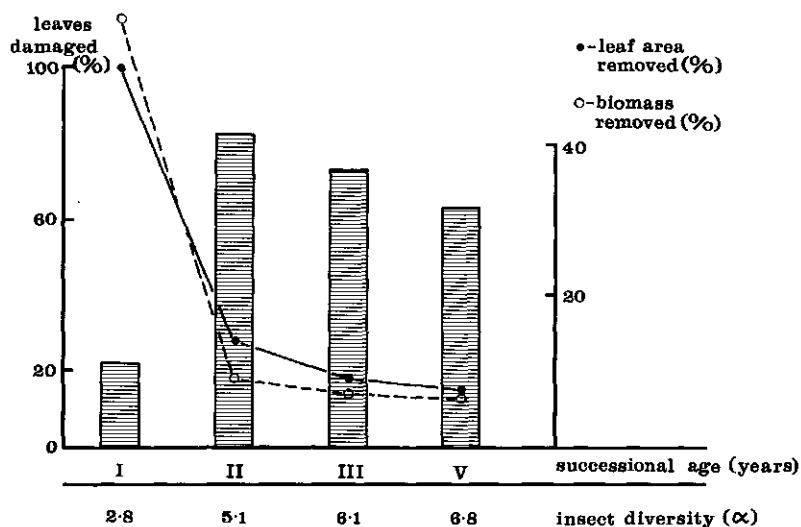2·8   5·1   6·1   6·8   insect diversity (∝)

Fig. 4. The effect of insect herbivores on *Trifolium repens* in habitats of different successional age. Based on four samples per season.

leaf area and biomass removed was much greater; thereby corroborating the suggestion that the apparent impact of insects is greater earlier in succession. Explanation for this may rest on differences in the structural complexity of the vegetation in relation to the successional age of the site. The early sites have a simpler structure which may favour the full exploitation of relatively few leaves.

Such measurements of damage emphasise the role of defoliators, and are also liable to question since the response of the plant is not taken into account. There are, however, two experimental approaches which are suitable to resolve whether animals are significant regulators of plant populations. One of these involves a deliberate reduction of the herbivorous insect load and the other a deliberate increase in the plant species in question, examples of the latter situation are well displayed in agriculture and forestry (Harper, 1977). The former approach is more appropriate to assess the role of insects in the development of a natural plant community. However, this approach has only rarely been applied and then to a particular plant species (Cantlon, 1969; Waloff & Richards, 1977). Recently, Clive Stinson (unpublished) has experimentally excluded insects from an early successional habitat, protected from rabbits and birds, by the regular use of a non-persistent insecticide. Only molluscs have been uncontrolled, though regular samples have indicated extremely low populations on the light sandy soil. Plants were sampled six times during the season and both the treatment and the sampling continued for the second season and a new experimental site created. Results cited here are based on the first season, a number of differences are already apparent. During the early part of the first season there is a more rapid accumulation of plant species in the insect-free area. Although the number of species in the control area is similar by the end of the season the percentage cover at the beginning and end of the season, is considerably
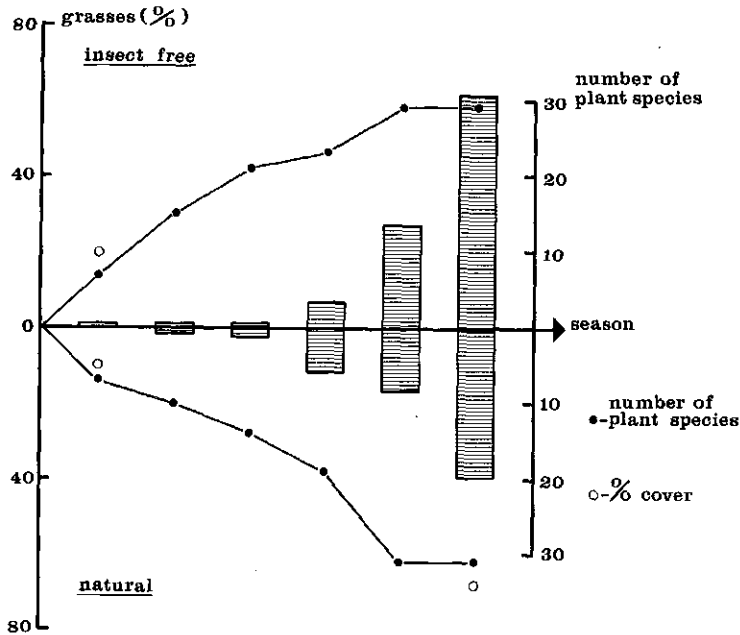
Fig. 5. Differences between a natural (control) early successional plant community and one from which insects have been experimentally excluded.

higher in the insect-free zone (19.2 and 83.9% compared with 10.8 and 68.7% cover) (Figure 5). The more rapid accumulation in the insect-free site may well be due to seedling success — there being 2.4 times as many seedlings in this site early in the season. Another difference is in the grass/herb index, expressed as the proportion of touches (point quadrats) of grasses to herbs, which is very much higher in the insect-free site. In fact a grass/herb index of over 60% is normally only reached in the third year of this succession (Brown & Southwood, in prep.). The difference in the index between the two sites becomes clear at a time when the grass-feeding insects increase rapidly in the control site (Figure 1).

The impact of insects on both the production and timing of reproductive structures is marked. The total number of reproductive structures is considerably higher in the insect-free site and there is also a difference in plant phenology with these structures appearing (and disappearing) earlier than in the control site. Figure 6 compares the number of touches of early reproductive structures for all plant species (flowering stem, pre-anthesis, anthesis) in the two sites. The difference is also seen when single species are considered; *Spergula arvensis* and *Raphanus raphanistrum* are two common ruderal species on these sites. The same trend was seen when fruiting structures are considered. Although the difference in the total number of these structures is not so marked. The full impact of differences in the number of reproductive structures has yet to be explored. The advance in the timing of flowering and fruiting results in a quicker turnover of species and may explain trends such as the difference in grass-herb index.
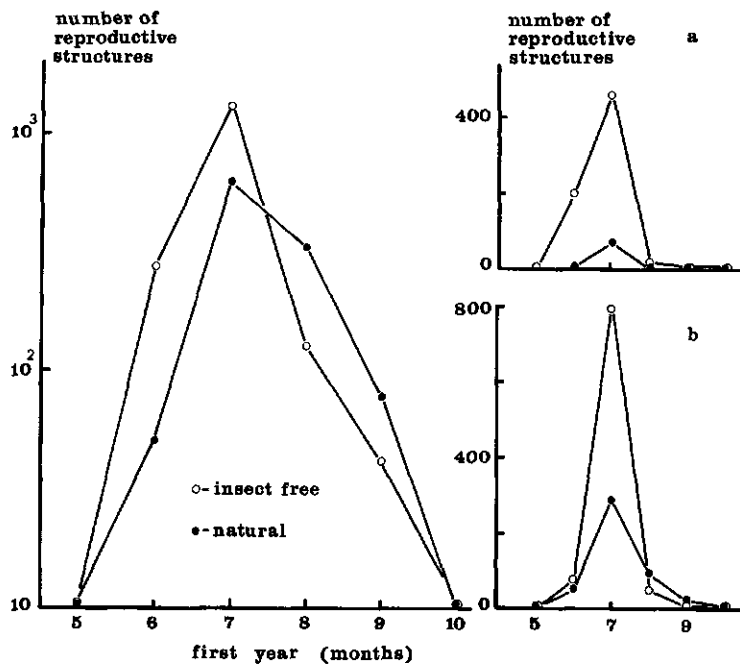
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Fig. 6. The effect of insect herbivores on the production of early reproductive structures of all species (left), (a) *Raphanus raphanistrum*, and (b) *Spergula arvensis*.

Conclusions

The impact of insect herbivores on early successional plant communities is considerable (Figure 7). An increased rate of plant species accumulation and turnover in the absence of herbivory has been demonstrated, the latter being associated with a forwards shift in the grass/herb index, a high seedling number early in the season, and a higher percentage cover of vegetation. There is also an overall increase in the number of reproductive structures and the timing of their production is advanced. In addition, there is evidence, to be presented elsewhere, that the biomass and structural complexity of the vegetation is greatly affected by the presence of insects — and other characters have yet to be explored.

All these features have been shown, under natural conditions, to have a characteristic rate in secondary succession (e.g. Southwood et al., 1979); the rate undoubtedly varying in different seres. The experimental removal of insect herbivores increases these rates. Thus the impact of the phytophagous insect community on early successional habitats appears to be a reduction in the rate of succession, forming an interesting similarity to the known effects of larger herbivores later in succession. The effects of insects on these later stages must be the next question to pose.
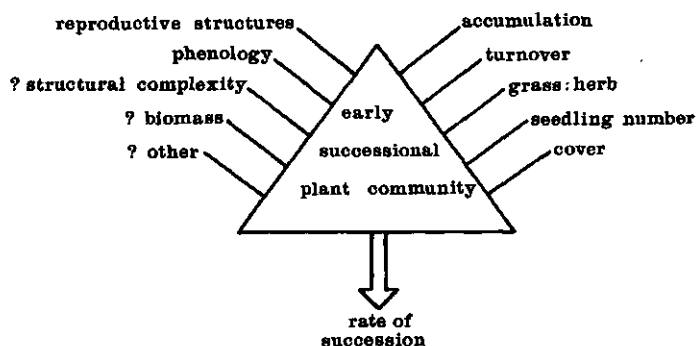
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reproductive structures — accumulation
phenology — turnover
? structural complexity — grass:herb
? biomass — seedling number
? other — cover

early successional plant community

↓ rate of succession

Fig. 7. Summary of the characteristics of an early successional plant community affected by insect herbivores.

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The influence of the dynamics of a population of herbivorous beetles on the development of vegetational patterns in a heathland system

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Abstract

Lochmaea suturalis is a monophagous herbivore, feeding on *Calluna vulgaris*. During outbreaks of this pest, *Calluna* plants frequently die off over large areas and are replaced by grasses. *Lochmaea* is able to survive the depletion of its food source through its capacity for dispersal by flight. Females show this capacity during early spring only, prior to oviposition. A shortage of food enhances the animal's disposition to flight and delays oviposition and the concomitant gradual breakdown of the wing muscles. During 1979-1980 the course of a local outbreak was followed. *Lochmaea* spread out over the plot in phases. Locally its mortality was very high, due to the entomopathogenic fungus *Beauveria bassiana*. These features of the outbreak were responsible for the development of a mosaic pattern in the vegetation. Food consumption and faecal production of *Lochmaea* were measured under semi-natural laboratory conditions. The measured rates of consumption and faecal production are discussed in relation to known data on energy flow in *Calluna* stands and about input of nutrients into heathlands.

Key-words: Lochmaea suturalis, Calluna vulgaris, Beauveria bassiana, outbreaks, dispersal, flight, vegetational patterns, population dynamics, oviposition

The heather beetle *Lochmaea suturalis* Thomson (Coleoptera: Chrysomelidae), has re-emerged as a pest of heather in the Netherlands since 1978. Earlier outbreaks this century were reported around 1927, 1945 and 1967 (Blankwaart, 1977). During outbreaks extensive areas of heather are affected and due to the damage caused by the beetles the heather turns brown. It often dies off as a result and grasses begin to dominate in the area that was formerly a heather stand, a big problem in nature conservation. Long-term botanical studies on heathlands by de Smidt (1977) have emphasized the importance of this type of damage by *Lochmaea* in heathland ecosystems in the Netherlands.

The heather beetle studies reported in this paper have been carried out on the heathlands 'Oud Reemst' (near Arnhem) and on 'Hoorneboegse heide' (near Hilversum) beginning in 1979.

Lochmaea suturalis

The heather beetle is a small beetle with a length of 6 mm. Cameron et al. (1944) and Schrier (1981) have described its biology and distribution. The species is strictly monophagous and its distribution is closely correlated with the distribution of *Calluna vulgaris* (L.) Hull in Western Europe. Outbreaks have been reported from Great Britain, Belgium, Denmark, Germany, Sweden, France, Switzerland and the Netherlands (Blankwaart, 1977).

In the spring adult beetles become active when the mean daily temperature rises above 9°C. They leave the litter layer and climb into *Calluna* plants. Local beetle density may be very high (up to 2000/m²), especially at the edges of areas that have had high infestations the previous year. In April and May beetles may swarm e.g., large numbers of flying heather beetles were observed in 1979 and in 1980 on the first calm, sunny days in spring when the mean daily temperature rose above 16°C (Figure 1) (van Schaick Zillesen & Brunsting, in prep.). The beetles were carried away by a gentle breeze, as *Lochmaea* seems unable to choose its direction of flight. The flight took place in sunny weather but as soon as a cloud obscured the sun, or the wind rose, the beetles landed. Many beetles die during these migrations. They have been seen landing in great numbers in towns, fields or on the surface of lakes etc. The period during which dispersal by flight occurs may last several weeks but similar wheather conditions occurring later in spring no longer cause the beetles to take flight.

Copulation takes place during the dispersal period and adults of this generation may survive until the end of June. Eggs are found in May and June and larvae from June until the end of August. From mid August onwards the adult beetles of the new generation emerge and increase in weight and develop flight muscles during late summer and autumn.

Autumn flights of the heather beetle have not been reported before, but in 1979 a period of intensive flight occurred in October. The weather conditions were similar to those during flights in spring, but were exceptional for the autumn. This may account for the absence of earlier records. Beetles retreat into the litter to hibernate in November, when temperatures drop below 9°C.

The course of an outbreak

The onset of an outbreak is marked by the appearance of brown areas measuring tens of square metres or more in fields of *Calluna*. These brown areas contain high densities of heather beetle larvae, sometimes as many as 1000/m². These larvae cause such heavy damage that the leaves die off, hence the brown appearance of the plants.

The new generation beetles walk to the edges of the foci in search of food, resulting in a high density of beetles around the edges of foci, up to 2000/m². This high density 'front' remains during winter, when beetles reside in the litter layer. This situation was found in the experimental plot on the heathland 'Oud Reemst' in April 1979 (Brunsting & Schrier, in prep.). Consecutive observations of the density of *Lochmaea* along a transect are presented in Figure 2, along with the degree of
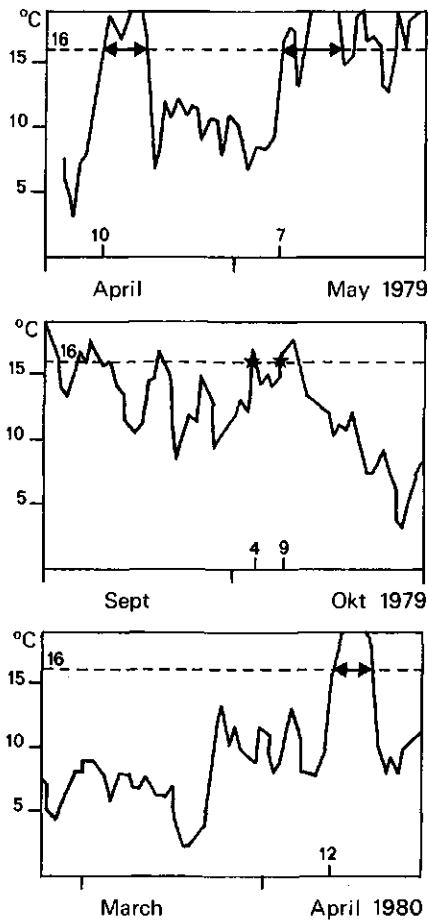
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Fig. 1. Dispersal flight (★ and ◄——►) of *Lochmaea suturalis* in relation to mean daily temperature.

damage to the *Calluna* plants as estimated by G.W. Heil (pers. comm.). At the focus (around A) of the previous year *Calluna* had died off as a result of the attack. Along the boundary of the focus a zone with high densities of beetles was found, but here the degree of damage to *Calluna* was only intermediate. Outside this zone only a few beetles were found and no damage to *Calluna* was observed.

During the flight period, all beetles left the focus and the rest of the area (Figure 2) was colonized more or less evenly. The high densities in the 'front' zone diminished (Figure 2, May 1979) and thus the 'front' disappeared. Many of the corpses (up to 285/m²) of *Lochmaea* left behind in the focus and the 'front' were covered by the entomopathogenic fungus *Beauveria bassiana* (Bals) Vuill which was found to be lethal to beetles and larvae in the laboratory (unpubl. data). In the 'front' area the plague failed to develop (Table 1) and *Calluna* was able to recover. Outside the
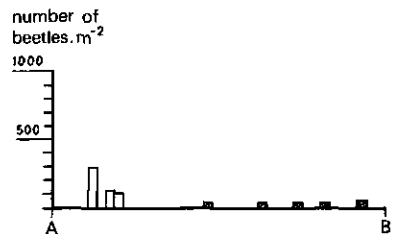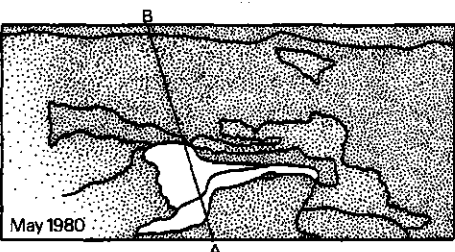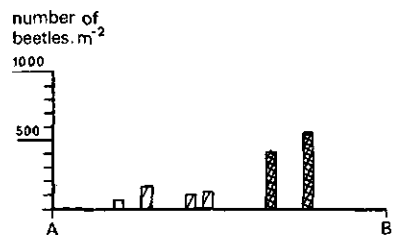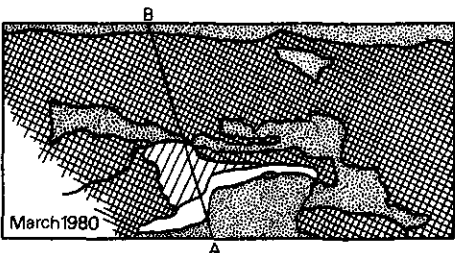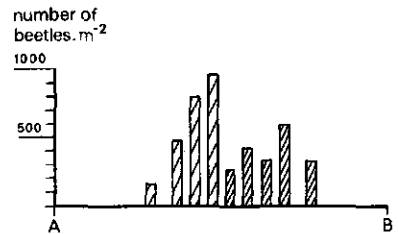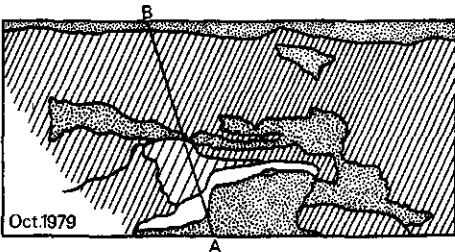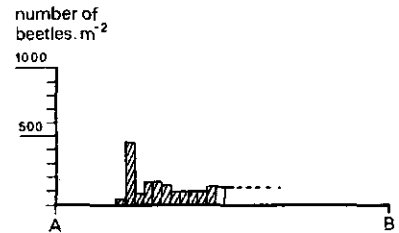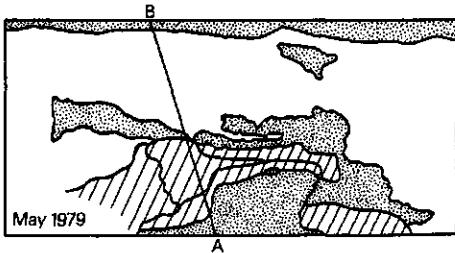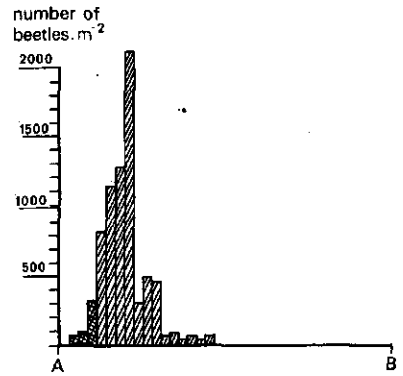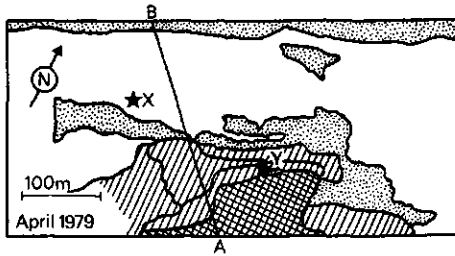
Fig. 2. Densities of adult *Lochmaea suturalis* along a transect AB in the heathland 'Oud Reemst' during the course of an outbreak, with corresponding changes in vegetational patterns.

Table 1. Changes in density (numbers/m^2) of *Lochmaea suturalis* in two plots X and Y (see Figure 2) on the heathland 'Oud Reemst' (Hoge Veluwe) during 1979 and part of 1980. *Beauveria bassiana* was active in plot Y.

|	Plot X	Plot Y	Period
Adults			
early spring	0	1300	start of April 1979
dispersal phase			April–May
oviposition phase	160	160	May–June
Eggs	5000	2900	June–August
Larvae	1000	200	June-August
Pupae	400	8	June-August
Adults			
new generation	375	0	August
dispersal phase			October
oviposition phase	25	170	May–June 1980

former 'front', however, the population of *Lochmaea* built up and the *Calluna* turned completely brown during late summer and died off in winter (Figure 2, March 1980).

During the flight periods in autumn 1979 and spring 1980 nearly all beetles left the area. A few beetles stayed or resettled in the former 'front' (Figure 2, May 1980) but the beetle population again failed to develop. As a result of the outbreak as a whole *Calluna* disappeared and grasses (*Deschampsia flexuosa*) became dominant over the whole area except in the former 'front', where *Calluna* recovered completely. This was still the situation in the autumn of 1981.

Estimates of the density of all stages of *Lochmaea* during the outbreak are given in Table 1. *Lochmaea* produced fewer eggs and larval mortality was higher in a plot (indicated by Y in Figure 2) inside the former 'front', where *Beauveria* was active, than in a plot (indicated by X in Figure 2) outside the 'front'.

The change in the vegetation from heather to a mosaic of heather and grasses was entirely due to the outbreak of *Lochmaea*, the dynamics of which determined the shape of the pattern. The dynamics of the heather beetle population itself was determined mainly by migration and mortality, the latter being due, at least in part, to an entomopathogenic fungus.

Flight capacity

Dispersal flight occurs during the first calm, sunny days in spring when the mean daily temperature rises above 16°C. After these flights the reproductive period starts, and the beetles do not fly any more, even under favourable conditions. From dissection it was concluded that wing muscles break down in females at the time of oviposition. Van Schaick Zillesen & Brunsting (in prep.) report that shortage of food influences oviposition and flight capacity. Beetles which were sampled in early spring and kept without food for 12 days flew frequently, whereas well-fed beetles rarely flew.

Table 2. Influence of food availability on egg production and size of wing muscles of *Lochmaea suturalis* females.

Period (weeks)	Abundant food		Shortage of food	
	eggs/day	diameter of flight muscle fibres (μm) ± S.D.	eggs/day	diameter of flight muscle fibres (μm) ± S.D.
0-1.5	0.0	77.4 ± 8.4	0.0	76.0 ± 9.4
1.5-3.5	0.6	66.1 ± 13.0	0.0	67.9 ± 13.5
3.5-5.5	0.9	49.4 ± 14.6**	0.0	67.5 ± 15.0**

** Significantly different at $P < 0.01$ (Wilcoxon).

In another experiment beetles caught in early spring were fed only half of the time, while the control group was fully fed. In the control group, females started oviposition and the volume of their wing muscles decreased, while females kept short of food did not lay eggs and the volume of their wing muscles was hardly reduced (Table 2). This oogenesis-flight syndrome has been reported in other insects (Johnson, 1969; Rankin, 1978; de Wilde, 1969).

The dispersal capacity of these beetles is not only dependent on the occurrence of suitable weather conditions during spring, but is also tuned to the availability of food. This tuning to food results in the beetles flying away from food-depleted areas but remaining in places where food is abundant. In this way *Lochmaea suturalis* escapes extinction after destroying large areas of its food plants.

Consumption, growth and faecal production and the ecosystem

Food consumption, growth and faecal production were estimated under simulated field conditions as far as day-length and temperature fluctuations (daily and seasonal) are concerned (Brunsting et al., in prep.). The measured rates are presented in Figure 3.

High consumption rates are found during oviposition in June, at the end of the larval stage in July and shortly after emergence in September; these high rates coincide with the production of eggs or a high rate of increase in the weight of the individuals. From these data it is estimated that one beetle between egg hatch and reproducing adult of 4.5 mg dry weight requires 151 mg of food (dry weight), of which 64 mg is assimilated and 87 mg excreted.

The total consumption and faecal production in a population of *Lochmaea* during an outbreak can be estimated from data such as those presented in Table 1 (plot X) and Figure 3. In Table 3 data are given per hectare. Preliminary data on nitrogen, which are based on the analysis of a small number of samples, are given in brackets. Some data from the literature on the biomass and input of nitrogen to *Calluna* and the impact of sheep grazing in a similar heathland are given for comparison.

The total food intake of the beetles is less than the potential amount of food available to them in the ecosystem and this consumption, at least in outbreak areas,
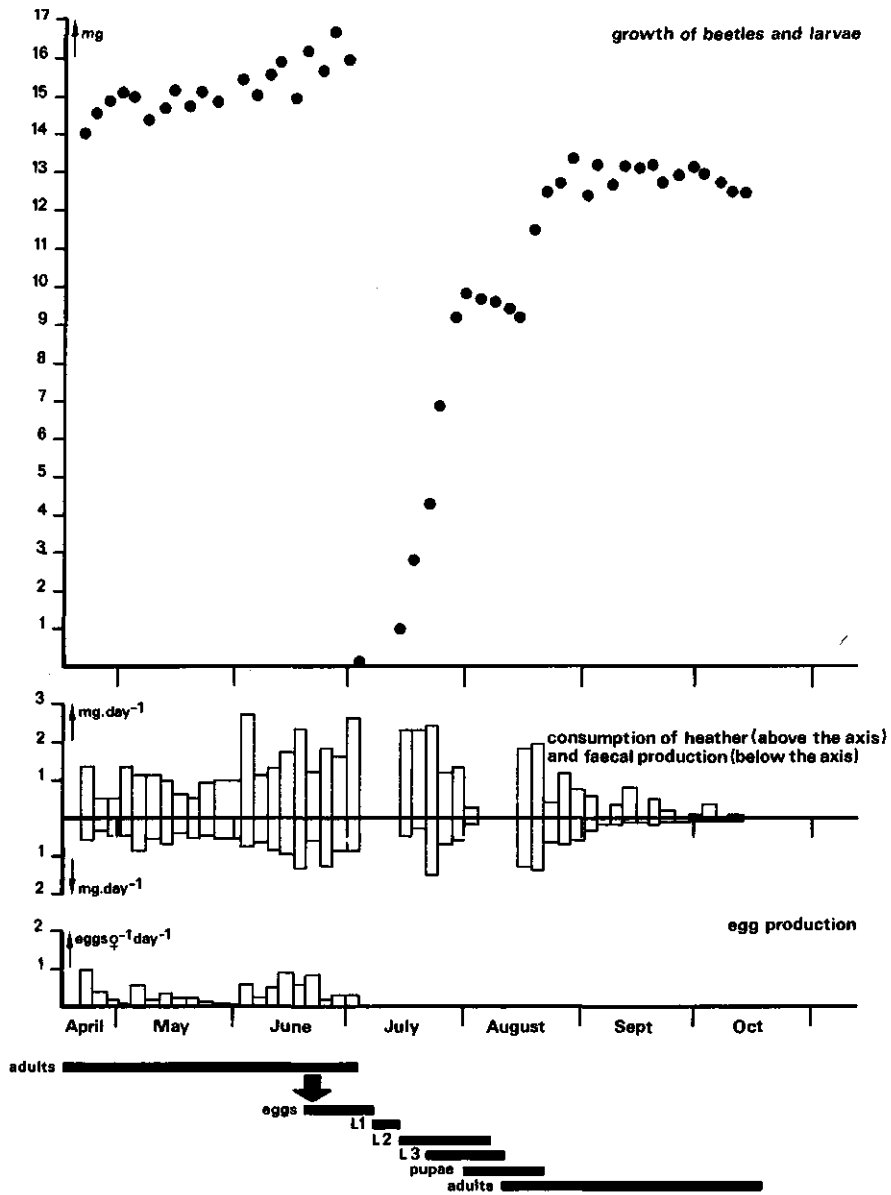
Fig. 3. Animal growth (fresh weight), food consumption (dry matter), faecal production (dry matter), and egg production of *Lochmaea suturalis* under simulated field conditions. Figures are in mg per individual per day.

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Table 3. Food consumption, faecal production and mortality of *Lochmaea suturalis* during an outbreak. For comparison some data on heather ecosystems are given (de Smidt, 1979; G.W. Heil, pers. comm.). All estimates are in kg dry matter per hectare; preliminary data on nitrogen are given in brackets.

	Consumption	Faecal production	Corpses
Adults, oviposition	82	52	7
Larvae	344[1]	132	13
Adults, pre-diapause	115	84	
Total	542 (8)	268 (5)	20 (2)
Data on heather ecosystems			
Standing crop (above ground)		10^4 (10^2)	
Green shoots		2.5×10^3 (30)	
Yearly input of N from the air		10	
Grazing by sheep per year		350 (6.6)	

1. Consumption of food is not always equal to the loss of *Calluna* plant material. Especially larvae of the third instar waste large amounts of food since many leaf particles fall on the ground.

exceeds that of sheep at normal stocking density (0.8 per ha). Nitrogen return to the soil by faeces and corpses is of the same order of magnitude as the yearly natural input from the air. This return to the soil is augmented by the decay and mineralization of the killed *Calluna* plants. The effects of this acceleration of the mineral cycles are not yet fully understood, but the outcome of experiments on the effect of added inorganic fertilizer on heather stands suggests that the increase in nitrogen in the soil is an important factor in the replacement of *Calluna* by grasses (G.W. Heil, pers. comm.).

Outbreaks of *Lochmaea suturalis* have a marked influence on the vegetational diversity of the heathland ecosystem by causing the death of *Calluna* over local areas and its replacement by grasses. This pattern is governed by the dynamics of the *Lochmaea* population where local food shortages lead to dispersal of the adult beetles from areas of high density and the subsequent formation of other local outbreak foci.

Acknowledgments

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Plant nutritional strategies and insect herbivore community dynamics

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Abstract

Plants from high nutrient status habitats have high growth rates, high tissue nutrient levels and rapid responses to changing soil nutrient concentrations. The insect herbivore community of these plants, e.g. *Holcus mollis*, is dominated by nitrogen specialist species which actively seek out patches of host plant of their preferred nutrient status, and are very responsive reproductively to plant nutrient levels. Plants from nutrient-poor habitats are unresponsive to soil nutrient levels and have low tissue nutrient levens and active secondary chemistry. The insect herbivore community of these plants is dominated by taxonomic specialists which are sedentary and relatively unresponsive to available nutrient levels in their host plant.

Key-words: population dynamics, nutrition, nitrogen, *Holcus mollis, Erica cinerea*, assimilation, Cicadellidae

Chapin (1980) has reviewed the contrasting nutritional strategies of plants from fertile habitats and those characteristic of less fertile situations. These nutritional differences will, in turn, affect the way in which the insect herbivores are able to exploit these species. This paper concentrates on aspects of nitrogen nutrition as this is the best understood area both botanically (Chapin, 1980; Chapin et al., 1980; Pate, 1982) and entomologically (McNeill & Southwood, 1978; Prestidge, 1982; Prestidge & McNeill, 1982a,b).

The basic differences between these two types of plant nutritional strategy, as seen by Chapin (1980) are summarised in Table 1. Stress-tolerant plants from low nutrient habitats characteristically have low tissue nitrogen levels, grow slowly and do not respond strongly or rapidly to changes in soil nutrient status. Plants of this type are also sensitive to the loss of valuable leaf material to herbivores and hence it has been suggested in the literature (Bryant & Kuropat, 1980; Chapin, 1980; McKey et al., 1978) that these plants should also show very high levels of anti-herbivore secondary substances (Feeny, 1976; Rhoades & Cates, 1976).

Competitive plants from nutrient-rich habitats, on the other hand, have high levels of leaf nitrogen, high growth rates and rapid responses to changes in nutrient availability. These plants ought, therefore, to be less sensitive to loss of leaf area to

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Table 1. Nutritional characteristics of plants of nu-
trient-rich habitats and nutrient-poor habitats (stress-
tolerant plants; Chapin, 1980).

Characteristics	High nutrient plants	Low nutrient plants
Nutrient absorption	rapid	slow
Growth rate	high	low
Tissue nutrient level	high	low
Metabolic rate	high	low
Nutrient losses	high	low
Secondary chemistry	relatively inactive	relatively active

herbivores and hence should show lower levels of anti-herbivore secondary sub-
stances.

We have been looking at the role of nitrogen in the relationships between the
grass *Holcus mollis* and its herbivores for a number of years and more recently we
have begun to look at several heathland plants in a similar way. *Holcus* shows many
of the characteristics of a plant from a relatively rich habitat and responds strongly
to the application of nitrogenous fertilisers and to variations between years in the
availability of nutrients in the soil, while the second group of plants i.e., *Ulex eu-
ropaeus, Ulex minor, Erica cinerea*, and *Calluna vulgaris*, in general show charac-
teristics of stress-tolerant plants.

Patterns of plant nutrient flow

Holcus mollis   The normal pattern of nitrogen availability to leaf feeders was in-
vestigated by looking at the total leaf nitrogen weekly throughout the growing sea-
son over a period of 8 years. The general pattern of total leaf nitrogen in the green
leaves through the season is shown in Figure 1. The spring flush foliage has a mean
peak total nitrogen content in dry matter of leaf of 38 mg/g and this drops rapidly
as the plant changes from somatic growth to reproductive growth to a summer low
of 15 mg/g. There was a rise in the autumn to an average content in dry matter in
the foliage of 37 mg/g associated with the initial growth of new shoots.

The amount of nitrogen available to the phloem feeding insects was assessed by
stripping the leaves from the grass stems and measuring the soluble nitrogen in the
stems. This method should give a measure of the nitrogen caught in transit in the
phloem. These data reveal at least three flows in a typical year (Figure 1). The first
is associated with the movement of nitrogen out of the leaves at the initiation of
flower development, the second is in mid-summer and is associated with seed pro-
duction and the third, which in some years is double, is associated with the with-
drawal of nutrients from the old shoots and its transfer to the new shoots which
begin their growth in the autumn.

The content of nitrogen in both leaves and stems is variable between years, prob-
ably due to variations in the rate of decomposition of the old shoots and losses due
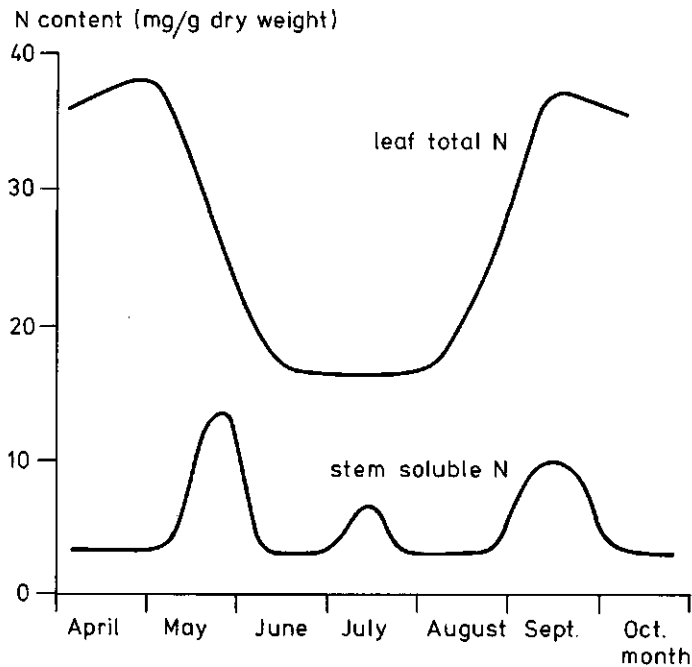
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N content (mg/g dry weight)

Fig. 1. The average patterns of total leaf nitrogen content in dry matter and soluble stem nitrogen content in dry matter throughout the season in *Holcus mollis* (1970-1978).

to leaching in the sandy soils of the experimental sites. The variation in spring leaf nitrogen content in dry matter is about three-fold (20-58 mg/g); the range for the other peaks in both stem and leaf is similar. The summer low contents in dry matter are less variable: 18-10 mg/g for leaves and 1.7-3.6 mg/g for stems.

Prestidge & McNeill (1982 a,b) derived a quality index for the available nitrogen based on the ratio between the amides (asparagine and glutamine) and their immediate derivatives, which are normally positive in their effect on insect performance (van Emden, 1973; McNeill & Southwood, 1978), and the 'non-protein' amino acids such as L-DOPA and γ-aminobutyric acid. This latter group of amino acids is normally associated with decreased performance in insects. The foliage and stems at times of flushing were consistently higher in quality than at other times of the year (Table 2). Years of high nitrogen contents in the plants tended to be of better quality than years of low nitrogen contents.

In *Holcus* the response to added nutrients was quite rapid with increased levels of nitrogen detectable in the leaves and stems within a week of the fertiliser application. The content of nitrogen continued to rise for another two weeks and then fell rapidly. The growth response was not seen until some five to six weeks after fertiliser application, well after the contents of nitrogen in the plant had begun to fall. The quality index of the foliage after the application of fertiliser showed that just as in a natural flush the increase in amino-nitrogen was largely as a result of increases in the amides. Thus the quality index rose during the artificially induced flush to

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Table 2. Amino-acid nitrogen quality index[1] in *Holcus mollis* leaves during high and low nitrogen flushes and the mid-summer low nitrogen period.

	Mean ± S.E.
High nitrogen flush	3.13 ± 0.75
Low nitrogen flush	1.50 ± 0.55
Summer low	0.89 ± 0.21

1. Amino-acid nitrogen quality index =
Σ amide group/(Σ non-protein group)
(Prestidge & McNeill, 1982 a,b).

fall again after the peak contents had been reached.

Ulex, Erica and Calluna   The content of nitrogen in the new shoots of all of these plants is generally below the equivalents in *Holcus*. They show a typical pattern of a spring peak in late May or early June followed by a slow fall throughout the summer in all species. The contents reached in late August or early September are maintained with little variation during the second and subsequent seasons. In *Erica*, for example, the contents of nitrogen, both total and soluble, are consistently well below the levels in *Holcus*, the spring flush (Figure 2) reaches a peak content of total nitrogen in dry matter of 10.6 mg/g — soluble nitrogen in dry matter 1.5 mg/g — falling to 6.7-7 mg/g for total and 0.8-0.9 mg/g for soluble in late August. The contents in dry matter in older wood in this species vary between only 5.5 and 6 mg/g for total (0.3-0.8 mg/g for soluble) during the season. The amino-acid quality index showed little consistent variation during the season remaining in the range 0.2 to 0.5 throughout the season, well below even the summer levels in *Holcus*.

Secondary chemistry in these species has an important role in reducing the availability of already scarce nitrogen (Feeny, 1970; Wint, 1982). A good example of the complexity of secondary chemistry in plants of this type is bracken fern (*Pteridium aquilinum*) extensively studied and reviewed by Lawton (1976, 1978). The listing of large numbers of potentially active classes of compounds however tells us little about the overall effect of these on the ability of the insects to extract the nitrogen from the plant. A better, though still not ideal, way of assessing the effect of these substances on the digestive processes of insects is to assay their effect on a standard enzyme system. A method for doing this using an amylase starch system was developed by Wint (1982) and was used to assess the effects of the secondary chemistry of *Erica*. The results showed that the secondary chemistry of *Erica* was extremely effective in delaying the action of the enzyme and were equivalent to 9.4% of foliage fresh weight in June rising to 21.3% fresh weight tannin equivalent by mid-August. These figures were much higher than any levels obtained by Wint for the foliage of non-stress-tolerant trees (Table 3).
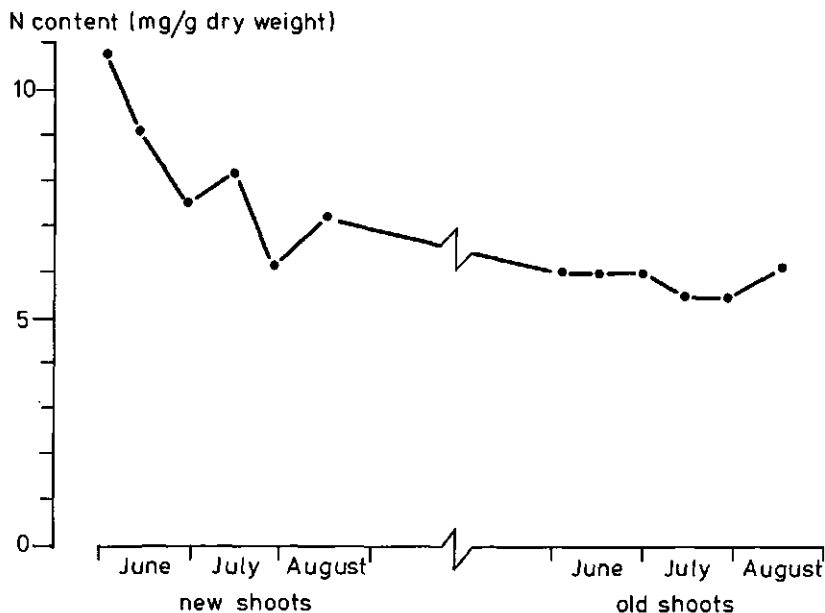
Fig. 2. The pattern of total nitrogen content in dry matter in new and old shoots of *Erica cinerea* throughout the season.

To examine the response of these plants to changes in nutrient availability, an area of heathland was treated with fertiliser at 30 g per m² ammonium nitrate equivalent in each of two seasons. The fertiliser was applied in May at the beginning of the major growth period of the heathland plants. Both the *Ulex* and *Erica* on the site showed a moderate response to these applications, the peak contents in dry matter of total nitrogen in *Erica* new shoots was increased to 14.1 mg/g and remained higher than the control areas throughout the summer, only reaching similar contents in mid-August. In the older material there was a peak content in dry

Table 3. Percentages of leaf wet weight oak tannin equivalents for the inhibition of amylase by leaf extracts of a stress-tolerant plant, *Erica cinerea*, compared with the data of Wint (1982) for deciduous tree foliage.

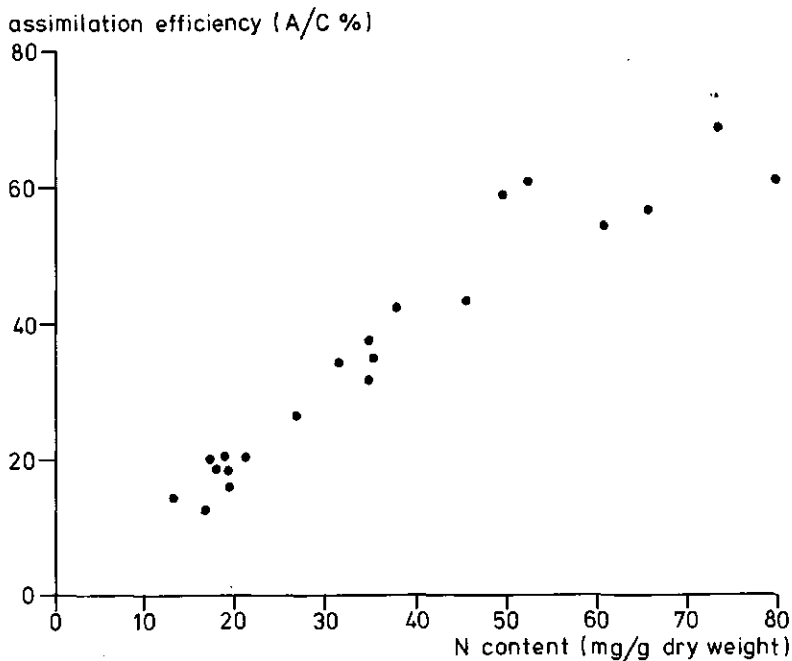| Plants	Range of value during the season
Erica	9.4% rising to 21.3%
Crataegus	3% rising to 10%
Corylus	< 1% rising to 4%
Fagus & Quercus	< 1% rising to 2%
Prunus & Malus	always < 1%

assimilation efficiency (A/C %)

Fig. 3. Energy assimilation efficiencies of *Leptopterna dolabrata* on food of different total nitrogen content.

matter at the beginning of June which reached 8.6 mg/g for total nitrogen, but this quickly dropped to the same content as in the equivalent tissues in plants from the control sites as the nitrogen was transported to the new growth. Patterns of change in soluble nitrogen showed similar changes on the fertilised plots.

The amino-acid quality index showed the greatest response of all in *Erica* reaching levels of between 0.8 and 1.0 in both new and old shoot material in early June, falling to the same level as the controls by late July or early August in both cases.

The insect herbivore community

Holcus mollis   There are some 40 species of herbivorous insects regularly associated with *Holcus mollis* on the experimental site, but only 24 of these occur at high enough densities to be studiable. The vast majority of these 24 species are leafhoppers, along with a species of thrip (*Aptinothrips rufus*), an aphid (*Holcaphis holci*), and several species of stenodeminid bugs.

The variation in available nitrogen in the grass, whether artificially or naturally induced, has little effect on the growth and nitrogen accumulation rates of the leafhopper nymphs. This is achieved by a compensatory increase in feeding rates on low nitrogen food, with a consequent though less rapid decrease in the assimilation efficiency as measured either in energy or nitrogen terms (McNeill & Southwood,

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Table 4. Energy assimilation efficiency and rates of nitrogen gain for two species of leafhopper on *Holcus mollis* (McNeill & Southwood, 1978).

	A/C (%)[1]	μg N gain per day	N content in dry matter of food (mg/g)
Dicranotropis hamata			
Early instars	24	1.1	18
Late instars	42	8.8	35
Adult female	43	17.3	38
Recilia coronifera			
Early instars	23	1.2	20
Late instars	21	11.2	18
Adult female	19	18.3	15

1. Assimilation/consumption.

1978) (Table 4). The nymphs of the Heteroptera show a similar pattern of compensation (Figure 3). Most species in this community have more than one generation per year and none takes more than one season per generation.

Unlike somatic growth, reproductive growth in almost all these species is very dependent on the concentration and quality of nitrogen available to the late nymphs and adults (Prestidge, 1982; Prestidge & McNeill, 1982 a,b; McNeill, 1973; McNeill & Southwood, 1978) showing a strong relationship to egg laying rate and total fecundity in the leafhoppers and to total fecundity in the Heteroptera. There is no effect on adult longevity or mortality, at least in the leafhoppers (Prestidge, 1982).

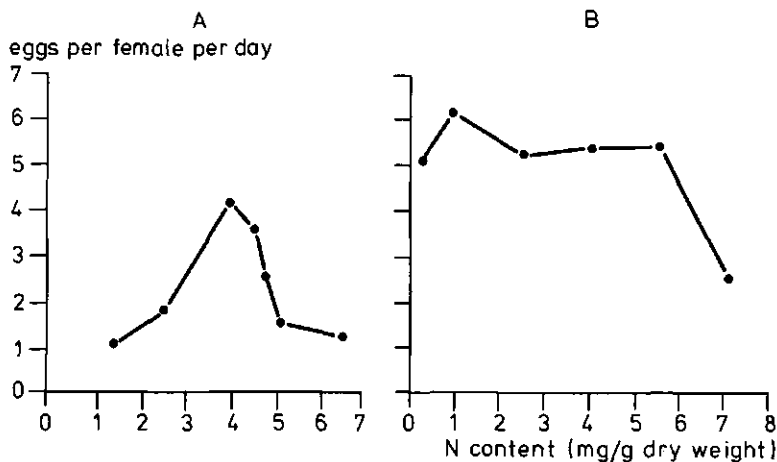In the majority of leafhopper species in this community there is a strongly peaked

Fig. 4. Egg laying rate and food nitrogen content in dry matter in (A) a nitrogen specialist leafhopper (*Ellymana sulphurella*), and (B) a taxonomic specialist leafhopper (*Dicranotropis hamata*).

Fig. 5. Typical results of a fertiliser application on (A) the nitrogen content of the plant, (B) the growth of the plant, and (C) the composition of the insect herbivore community in a grass sward.

relationship between egg laying rate and available nitrogen in the food plant (Figure 4). This means that most leafhoppers can only breed successfully over a relatively small range of plant nitrogen concentrations. These nitrogen specialist species are those that show a wide range of host plants (Prestidge & McNeill, 1982 a,b), and are all highly mobile species with well developed wings. These species appear rapidly on areas that have been fertilised as soon as the increased levels of nitrogen appear in the leaves and stems of the grass, and before there has been any detecta-

ble increase in the growth of the grass (Figure 5). It is these species that contribute to the high populations that occur on the fertilised plots later in the season as the nymphs of the next generation hatch.

A minority of the leafhopper species in the *Holcus* community are taxonomic specialists rather than nitrogen specialists. These species show a very different pattern of relationships to their host plant. The egg laying rate in these species is relatively little affected by changes in the levels of available nitrogen in the host plant and hence the relationship between the two is very flat over almost the whole range of plant nitrogen concentration values tested (Figure 4). Most of these species are less mobile than the nitrogen specialists and do not respond to fertiliser application in the same way. They are often species with reduced wings which are not capable of flight.

Ulex, Erica and Calluna   The herbivore community of these plants differs from that of *Holcus* in that it is characterised by a relatively small number of species and characteristically low population densities. A typical sample of 100 sweeps in June in the *Holcus* community produces 1500 to 2000 insects belonging to 15-20 species while the same sampling effort in an *Erica* community will produce only 150-270 insects belonging to 6 species.

The majority of insects in this simpler community are taxonomic specialists confined to one or a very few closely related species of plant (Hill, p.237). We know little, as yet, of their relationships to plant nitrogen in terms of reproductive effort, but preliminary work suggests that they resemble the taxonomic specialists on *Holcus* and are little affected by changes in host plant nutrient levels.

Many of them are very sedentary, with very few having more than one generation in a season and many requiring more than one season to complete their life cycle. A good example of a typical insect in this type of herbivore community is the gorse tingid *Dictyonota strichnocera*, which Brown (1981) found not to move from one bush throughout its life cycle; indeed few moved from the branch on which they were hatched.

Preliminary results from the fertilised heathland plots indicate that there is little if any of the movement onto fertilised plants and subsequent large population increases seen in the grass system. There are indications that there are slightly more insects on *Erica* in the fertilised areas but the differences are not significant. Samples taken on five occasions through the summer gave an average insect density of 234 per 100 sweeps on the fertilised plots and 190 per 100 sweeps on the control plots.

Discussion and conclusion

Plants of relatively nutrient-rich habitats, like *Holcus mollis*, have a nutritional strategy that is extremely responsive to changes in available nutrients, whether natural or artificially induced. They have a fast growth rate, high tissue nutrient levels and are efficient at absorbing soil nutrients. There is, hence, a large variation between local patches of habitat in nutritional status and plant phenology, within and between seasons.

The insect herbivore community in this case is dominated by species which exploit this variability by being taxonomic generalists, but nutrient specialists, in their host plant requirements. They are normally fast-growing, highly mobile species which depend on finding local patches of vegetation of the correct nutrient status for successful reproduction.

The relatively large-scale variations in nutrient status between seasons in one place means that most species of insect herbivore will also appear to change greatly in relative abundance from year to year when looked at on a small scale, giving the impression of an extremely labile community structure on host plants of this type.

Stress-tolerant plants, like many heathland plants, live in habitats where available nutrients are in short supply. These plants are relatively unresponsive to changes in nutrient availability, and are normally slow-growing with low tissue nutrient levels, inefficient at absorbing soil nutrients and have high levels of anti-herbivore secondary chemicals. The scale of year-to-year and patch-to-patch variation in plant nutrient status and phenology is thus much less.

The insect herbivore community on these plants is dominated by slow-growing taxonomic specialists that are nutrient generalists. These insects do not tend to move much from plant to plant and do not respond rapidly to natural or artificially induced changes in plant nutrient status. Looked at on a local scale, therefore, these insect communities appear to be stable with an order of relative abundance between species that does not change much between seasons.

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Seasonal patterns of phytophage activity on gorse (Ulex europaeus), and host plant quality

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Abstract

The effect of host plant influences, especially nutritive value, on the seasonal occurrence of the phytophagous fauna of *Ulex europaeus* was studied. Peak diversity in foliage-feeding insects occurred in spring and coincided strongly with peak foliage nitrogen content. Total energy content and soluble carbohydrate content did not vary seasonally, but remained relatively high all year. The structure of the community was therefore affected by food quality as measured by nitrogen content, or by some other factor closely correlated with it. Other factors are being investigated.

Key-words: insect diversity, *Ulex* species, nitrogen, carbohydrates, nutrition, population dynamics

A number of recent papers have pointed out the importance of variation in host plant influences on the structure of phytophagous insect communities through the year (McNeill & Southwood, 1978; Gilbert, 1979; Strong, 1979; Mattson, 1980). In particular, recent studies on bracken have shown how such influences may operate to constrain the size and diversity of the phytophagous fauna (Lawton, 1976, 1978; Rigby & Lawton, 1981). Most authors acknowledge variation in food availability as an important factor either in terms of changes in the absolute amount of accessible food or changes in the availability of that food. This view is almost inevitable considering the large seasonal variation in food quality found in many plants (McNeill & Southwood, 1978; Prestidge & McNeill, 1982) and the fact that food-quality, particularly protein content, is often limiting for insect development (Mattson, 1980).

The insect fauna associated with gorse (*Ulex europaeus* L.) in Europe has been reviewed by Zwölfer (1964). Recently a study began in the south of England to determine whether the seasonality and abundance of the foliage-feeding insects attacking this woody shrub were related to the nutritional quality of the host plant, estimated from measurement of soluble carbohydrate content, calorific value and in

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particular nitrogen content of the foliage. This involved systematic sampling of the insects associated with gorse for 3 years at two sites, with concurrent monitoring of host plant quality. The first results of part of this study are presented here.

Materials and methods

In 1979, 1980 and 1981 insects were sampled at two sites, Windsor Great Park (WGP) and Yateley Common (YC), from early spring to late summer. Fifteen samples were chosen haphazardly at arms length from a random co-ordinate, and each consisted of an estimated 200 g fresh weight of green foliage. Though judged visually, this measure proved consistent, and was checked regularly. Insects were beaten from the sample onto a 1-m² cloth tray, collected or counted, and the abundance of each species was corrected for differences in beating efficiency. Those species which occurred in fewer than 1% of all samples in 1980-1981 were regarded as vagrants and ignored unless previously recorded from gorse. The Berger-Parker dominance index was calculated for each sampling occasion (Southwood, 1978), and the seasonal variation of phytophage diversity is represented in Figure 1 as one minus this index.

On each sampling occasion in spring, three stems bearing many small new shoots were chosen haphazardly from each of three random co-ordinates. These were stored in the dark, and placed in a freeze-drier within 40 min. After drying, the new shoots were separated from old foliage, both were ground to powder in a ball-mill, and stored separately at −18°C. Later in the season three random new shoots provided sufficient material for analysis. This change in sampling technique did not affect the results obtained.

The calorific content of the foliage was determined using a Gallenkamp ballistic bomb calorimeter. Soluble carbohydrate content was measured as an indicator of simple carbohydrate levels in the foliage. The standard anthrone technique was used (Allen, 1974). Soluble nitrogen compounds were extracted from 100-mg samples of ground foliage with 2 ml of 5% trichloroacetic acid. Both total and soluble nitrogen levels were determined using the micro-Kjehldahl technique. Samples were digested by concentrated sulphuric acid with 1 g Na_2SO_4 and 0.05 g of selenium as catalyst. Digest analysis was by Technicon autoanalyser (Varley, 1966).

Results

Seasonal dynamics of the phytophagous fauna   The Berger-Parker dominance index was chosen to represent diversity as it is considered more robust than most indices (May, 1976; Southwood, 1978). Diversity of foliage-feeding insects in general was maintained throughout the sampling period, though it reached a peak in July. A large part of the fauna consisted of adult insects (mainly weevils) which fed little if at all, and whose larvae fed elsewhere. Figure 1 shows the variation of seasonal diversity of only those insects feeding on gorse as immature stages. Greatest diversity (i.e. minimum dominance) occurred in early spring, and then declined to a low level by the end of July. Both abundance and species richness peaked in early spring. In general, no new foliage-feeding species appeared on gorse after mid-June,
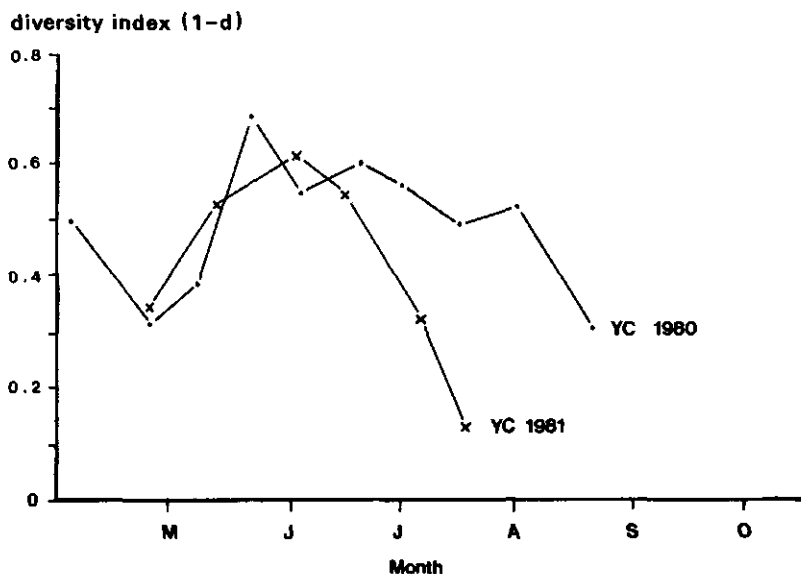
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diversity index (1-d)

Fig. 1. Seasonal variation of diversity of folivorous insects developing on *Ulex europaeus* at Yateley Common, as represented by the complement of the Berger-Parker Dominance index.

and almost all had completed larval or nymphal development by mid-July. Those species feeding on the reproductive parts of gorse all completed development before reproductive activity ceased in June.

Seasonal variation in the nutritional quality of foliage The texture of gorse foliage varied immensely from soft and succulent actively growing tissue to very tough and fibrous mature foliage. Vegetative bud-burst occurred in late May, and apical elongation of shoots continued throughout summer. Since shoots grew continuously, the terminal portion of all new growth appeared to be edible throughout the sampling period. By August the previous year's growth had become senescent, and all green foliage was therefore current seasons growth.

The total calorific content of gorse foliage in dry matter was approximately 23.8 kJ/g and there was no seasonal variation. This is a high energy content compared with figures for other plant foliage (Southwood, 1972).

Levels of soluble carbohydrate content also remained relatively constant, though in spring levels were slightly higher than later in the season (Figure 2).

Figure 3 shows the seasonal variation of total nitrogen content in new and old foliage at Yateley Common in 1979 and 1980. Nitrogen content in dry matter of foliage fell from a peak of 42 g/kg (protein content in dry matter of foliage 263 g/kg) immediately after new growth began, to approximately 18 g/kg (protein content 113 g/kg) after only 6 weeks. This low level was maintained for the remainder of the year. The same pattern was seen in levels of soluble nitrogen content, which dropped from 6 g/kg to 2 g/kg over the same period. Almost identical patterns were
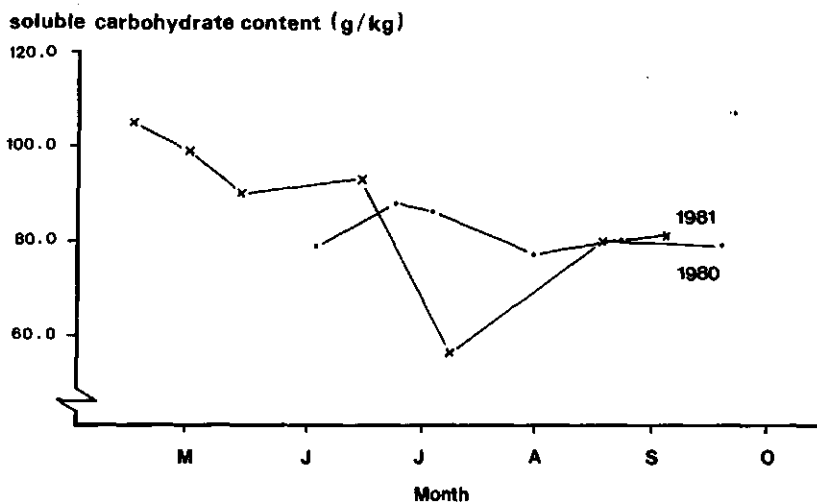
soluble carbohydrate content (g/kg)

Fig. 2. Seasonal variation of the soluble carbohydrate content in dry matter of *Ulex europaeus* foliage at Yateley Common.
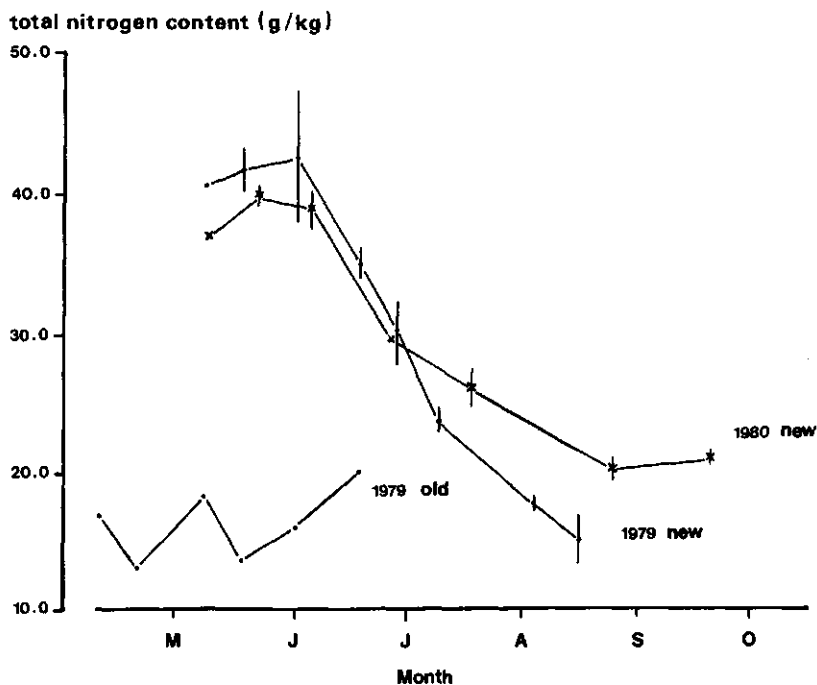
total nitrogen content (g/kg)

Fig. 3. Seasonal variation of total nitrogen content in dry matter of old and new *Ulex europaeus* foliage at Yateley Common.

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obtained in 1981, in gorse foliage from other sites, and even in another species, *Ulex minor* R. There was no significant difference in nitrogen content between different parts of the shoot.

The observed nitrogen levels were lower than those normally associated with an angiosperm, especially one which fixed nitrogen (Mattson, 1980). In a recent review, Chapin (1980) listed low nitrogen content, dilution of nutrient concentration with growth, and relatively high levels of non-structural carbohydrate content as characteristics of stress-tolerant species. *Ulex* spp. normally grow in acid infertile conditions, and share these characteristics with other heathland species. Characterisation of *Ulex europaeus* as a stress-tolerant species is reinforced by its growth patterns, evergreen habit, reduction in photosynthetic ability, and in particular, lack of response to high nutrient availability, which is discussed elsewhere in this volume (McNeill & Prestidge, p.225).

Conclusion

The seasonal occurrence of those species attacking the reproductive parts of gorse was governed entirely by the presence or absence of buds, flowers or pods, and this part of the phytophagous fauna has not been considered in detail here. On the other hand, gorse supported apparently edible green foliage throughout summer, and yet the occurrence of foliage-feeding species through the season was far from even. What therefore controlled the seasonality of the foliage-feeding fauna on gorse? Although the literature records a very high proportion of generalist species among gorse phytophages (Zwölfer, 1964), such records are notoriously misleading. Those generalist species which did occur in the study were rare, and contributed little to the overall structure of the phytophagous community. Seasonal patterns of diversity were therefore dominated by specialist gorse-feeding species.

The structure of the folivorous community on gorse, estimated using a diversity index, appeared to be dominated by seasonal variation in the availability of nitrogenous compounds in gorse foliage, or by some factor strongly correlated with total nitrogen content. Only nine species were commonly found developing on gorse, and peak abundance of five of these occurred in June, at the peak of nitrogen concentration.

The nutrient content of gorse foliage and the structure of the associated fauna appear to be stable from year to year, and from site to site. This could be predicted from the characteristics of such stress-tolerant plants as *Ulex europaeus* (Chapin, 1980; Mattson, 1980) and also from the prediction of Lawton and McNeill that insects which attack perennial long-lived plants should exhibit lower r-values than those attacking ephemeral species (Lawton & McNeill, 1979). They also predicted that such plants should be heavily protected by quantitative defences. The effects of gorse secondary chemistry and other plant-borne factors on the fauna on *Ulex europaeus* are also being examined at present. Attempts have been made to perturb this stable insect-plant relationship by fertilisation treatment, and these are mentioned by McNeill & Prestidge (p.225).

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The energy economy of fluid-feeding herbivorous insects

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Abstract

The performance of aphids is expressed in energy terms by the ratio of production to consumption (P/C) and this is controlled by the ratios of assimilation to consumption (A/C) and of production to assimilation (P/A). *Aphis fabae* and *Megoura viciae* living on bean stems have a P/C ratio 79% higher than leaf feeders, achieved by a higher A/C ratio. Aphids inhabiting galls have a P/C ratio 71% higher than similar aphids feeding on un-galled leaves, also achieved by a higher A/C ratio. It is suggested that A/C ratios are directly controlled by the availability of amino-nitrogen in phloem sap. Spittlebugs have a lower P/C ratio than predicted from their food because they utilize considerable energy in spittle production. Specialist fluid feeders have P/C ratios 80% higher than leaf chewing insects and this is achieved by higher A/C ratios (higher food quality) and higher P/A ratios (lower maintenance costs). A relationship between aphid adult weight and estimates of sap ingestion is explored.

Key-words: Aphididae, Cercopidae, Miridae, nutrition, consumption, assimilation, reproduction, growth, galls

Much of the stimulus for insect energy budget construction arose from the interest developed in ecological energetics by Lindeman (1942) and its elaboration by Odum (1957) and others. Their aim was to describe ecosystem function in terms of community energy flow and to do this with accuracy, energy budgets for individuals and populations are required. Although early work was encouraging and led to the formation of a coordinated scheme of world-wide cooperation, the International Biological Programme, it became clear that many factors influencing ecosystem function were not easily incorporated into energy models. Ecologists have lost interest in energetics but energy budgets for individual organisms provide a framework from which to hang a variety of concepts and factors which influence animal performance. The value of energy budgets is the ease with which comparison can be made between different individuals, populations and species but their principal drawback is the time and effort required for construction.

The animal energy budget was the form: $C = P + R + U + F$, where C is energy consumed (food intake), P is production (body growth and reproduction), R is metabolic heat loss (expended in activity, body maintenance, cellular biochemis-

try etc.), U is the energy contained in nitrogenous excretory products, and F is the energy in faeces. P + R + U equal A, the energy passed across the animal intestine wall and assimilated into the body. Every energy budget is unique, the values for different pathways depending upon numerous, often interacting variables and consequently most comparison is done by calculating ratios of different energy expenditures. These ratios are 'efficiencies' and indicate the use of energy by the animal for the production of animal biomass. The ratio of P to C (often called the growth efficiency) shows the overall efficiency of the animal. For a well fed individual in the laboratory this will reflect its physiology but for a population in the field extrinsic factors such as predation and food availability will also operate.

The ratio P/C depends entirely on the value of two other energy ratios, A/C and P/A. The A/C ratio reflects the energy which is available for all metabolic processes, including production, and is affected by the quality of food, the amount of protein for instance, or by the body demand for carbohydrate or protein. The P/A ratio indicates the amount of assimilated energy which is channelled to production and depends on other demands for assimilated energy, the amount lost as heat during respiration (R) and contained in U. The higher the P/A ratio the less energy has been 'spent' in supporting the production system.

Energy budgets for plant fluid feeding insects

There are energy budgets for 13 aphids and one jassid, all phloem sap feeders, for one spittlebug, a xylem sap feeder, and for one mirid feeding on mesophyll cell contents. In an extensive survey of animal energy budgets Schroeder (1981) reported 19 for leaf chewing insects. The aim of this study is to evaluate energy budgets as an aid to understanding the relationship between plant fluid feeding insect performance and the host food plant.

Energy budgets for the following insects are used. Aphids: *Aphis fabae* (Llewellyn & Qureshi, 1978), *Megoura viciae* (Llewellyn & Qureshi, 1979), *Acyrthosiphon pisum* (Randolph et al., 1975), *Rhopalosiphum padi* (Dixon, 1971), *Macrosiphum euphorbiae* (Llewellyn & Hargreaves, unpubl.), *Aphis pomi* (Hargreaves, 1979), *Dysaphis devecta* (Hargreaves, 1979), *Eucallipterus tiliae* (Llewellyn, 1972), *Drepanosiphum platanoidis* (Dixon, 1970), *Tuberolachnus salignus* (Llewellyn et al., 1974; Hargreaves & Llewellyn, 1978; Llewellyn, unpubl.), *Macrosiphum liriodendri* (Van Hook et al., 1980). Spittlebugs: *Philaenus spumarius* (Wiegert, 1964a). Mirids: *Leptopterna dolabrata* (McNeill, 1971). Jassids: *Cicadella viridis* (Andrzejewska, 1967). Unfortunately almost the only common feature of these energy budgets is their expression in calories or joules and in order to make the data comparable some recalculation has been necessary. I have used energy budgets for individual aphids calculated from the day of birth to the day of death. In a few cases this was not possible, the energy budgets for *D. platanoidis* (Dixon, 1970) for instance, were reported only as energy ratios.

Aphid energy budgets Aphid energy budgets are the largest group so I will first assess their contribution to aphid biology. Almost every environmental factor investigated seems to influence aphid performance in some way and this is reflected in
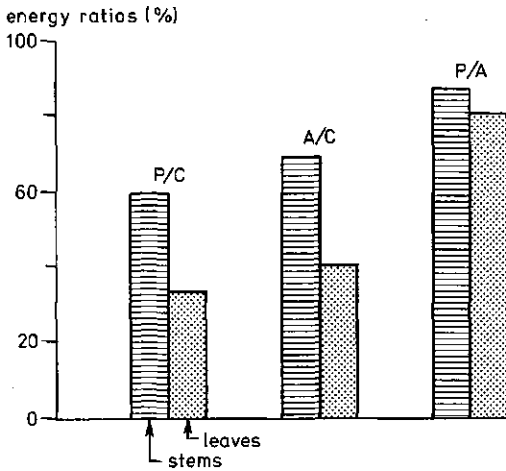
energy ratios (%)

Fig. 1. Energy ratios of *Aphis fabae* and *Megoura viciae* feeding on the leaves and stems of broad bean seedlings. P: production; C: consumption; A: assimilation. See text for further explanation.

the energy budgets. In Figure 1 the energy ratios P/C, A/C and P/A for *A. fabae* and *M. viciae* feeding on different parts of broad bean seedlings are shown. The P/C ratio shows that stem-feeding aphids perform substantially better (an increase of 79%) than their leaf dwelling counterparts. Metabolic costs (P/A ratio) are similar so the difference is due to different assimilation (A/C). Stem feeders assimilate al-
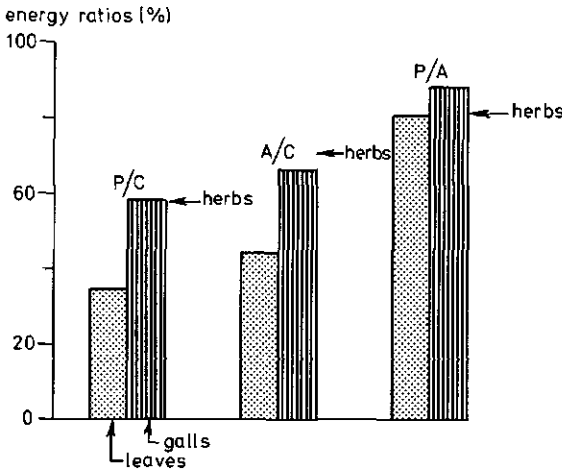

energy ratios (%)

Fig. 2. Energy ratios of *Dysaphis devecta*, *Aphis pomi* and *Macrosiphum euphorbiae* living in galled apple seedling leaves and of *M. euphorbiae* and *A. pomi* living on ungalled apple seedling leaves. Arrows indicate energy ratios of aphids feeding on herbaceous plants. See Figure 1 for further explanation.

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most 70% of their energy consumption compared to 40% in leaf feeders.

Kennedy (1951) and Forrest (1971) concluded that aphids obtained a nutritional advantage from galling plant tissue but the precise quantitative advantage and its relationship to food quality has not yet been elucidated. Both *D. devecta* and *A. pomi* induce leaf rolling of young apple seedling leaves and Figure 2 shows the energy ratios of these aphids and of *M. euphorbiae* living in galls and compares them with *A. pomi* and *M. euphorbiae* living in ungalled leaves of a similar age. (*D. devecta* will not live on ungalled leaf tissue). Aphids feeding in galls have a P/C ratio 71% higher than similar aphids feeding on leaves and their overall performance is similar to that of aphids feeding on herbaceous plants (Figure 3). P/A ratios for gall feeders are the highest recorded for any fluid-feeding insect and although only 8% higher than the mean for aphids feeding on ungalled apple seedling leaves they suggest some advantage in addition to the nutritional benefit. The bulk of the difference in P/C ratios is generated by the A/C ratio however with gall-feeding aphids able to assimilate 48% more of their energy intake. The increase in the A/C ratio for stem-feeding *A. fabae* and *M. viciae* over their leaf-feeding counterparts was 73% which suggests that the nutritional advantages of galling may be less than those already available in existing plant structures.

Differences in the performance of aphids living on trees and herbaceous plants are usually attributed to different amounts of amino-nitrogen being available in the phloem sap (Dixon, 1975) and Figure 3 shows energy ratios for aphids feeding on different types of plant and tissue. Although Hargreaves (1979) reported a P/C ratio of 74% for *D. devecta* living in galls the average for aphids feeding on herbaceous plants and in galls is almost the same and a P/C ratio of 58% probably represents a realistic maximum for aphids. Tree dwelling species have an average P/C ratio of
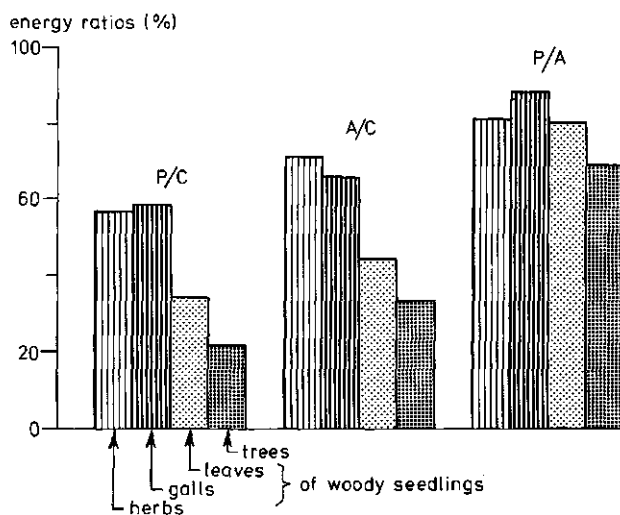

Fig. 3. Energy ratios of aphids feeding from different types of plants and plant tissues. See Figure 1 for further explanation.

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20%, rather higher than the 5% suggested by Dixon (1975). This is so because of values of 44% for *T. salignus* (Llewellyn, unpubl.) and 27% for *M. liriodendri* (Van Hook et al., 1980). A/C ratios exhibit a similar pattern suggesting that it is indeed the chemical composition of phloem sap of different plant types which is responsible for the variation in P/C ratios.

Much of the information on phloem sap composition is for angiosperm trees because a simple incision into the bark produces an exudate which can be analysed. Unfortunately, it does not work for most herbaceous plants (Ziegler, 1975). An extensive list of sugars found in angiosperm tree sap (Zimmermann & Ziegler, 1975) shows that sucrose is the commonest sieve tube carbohydrate with concentrations of between 10 and 20 mg/ml but in some species raffinose may be substituted and D-mannitol and sorbitol may occur in significant amounts. There is no evidence that A/C ratios in aphids may be mediated by carbohydrate however so we must look to amino-nitrogen. Quantitative data on nitrogen content of phloem sap is extremely sparse and available only for angiosperm trees where Ziegler (1975) suggested it to be in the order of 1 mg/ml. Kollmann et al. (1970) reported 9.8 mg/ml protein for *Cucurbita maxima*, largely consisting of P-protein and therefore unlikely to be available to aphids. Dadd (1973) listed the amino acids necessary for aphid growth and the evidence that aphids can utilize other nitrogenous compounds is rather sparse (Leckstein & Llewellyn, 1974). It seems likely that A/C ratios will be linked to amino-nitrogen availability in the phloem sap but there is yet no method to test this relationship. Given the considerable stability of the P/A ratio (a mean of 77% for aphids) it follows that changes in P/C will mirror the changes in A/C and perhaps these ratios should be used as an index of phloem sap quality which is both more broadly based and more easily compared than either aphid growth rate or fecundity.

Aphids and other plant fluid feeders   Energy ratios can be used to compare performance of insects with similar and different feeding styles. Amongst the plant sap sucking insects phloem feeders predominate but there are also insects which utilize xylem sap and mesophyll cell contents and their energy ratios are compared with aphids and jassids in Figure 4. The mean energy ratios for 19 species of leaf chewing insects are also included. The principal energy and nitrogen source in xylem sap is probably amino acids (Wiegert, 1964b) and consequently we would expect high A/C ratios, with most of the amino acid being absorbed. Wiegert (1964a) estimated that 10% of amino acids ingested were not assimilated and this yields an A/C ratio of 90%. The striking feature of spittlebug energetics however is the exceedingly low P/A ratio, indicating that little of the assimilated energy, all of which is chemically suitable for growth and reproduction, is actually channelled to production. A closer look shows that 59% of assimilated energy was used in respiration (as opposed to 24% in aphids) and a further 36% was contained in organic material used to produce the protective spittle. Although *P. spumarius* has a food source able to sustain a high P/C ratio this does not occur because of high maintenance and protection costs. The mirid *L. dolabrata* ingests the contents of leaf mesophyll or ovule cells and from its A/C ratio we can suggest the nitrogen content of this food is rather low. The P/A ratio is however lower than the mean for aphids indicating that
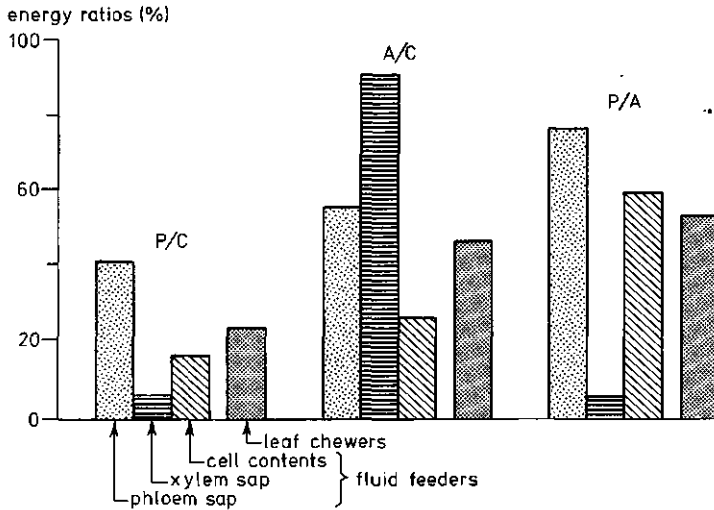
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energy ratios (%)

Fig. 4. Energy ratios of phloem sap feeders, xylem sap feeders, mesophyll cell content feeders and leaf chewing insects. See Figure 1 for further explanation.

respiration costs are higher, possibly because growth is slowed by nitrogen shortage and a substantial biomass is turning over slowly. The generalists among phytophagous insects, the leaf chewers, have intermediate energy ratios and this is the standard against which we can assess the effectiveness of the sap-sucking specialists. Mean P/C ratios for aphids are almost 80% higher and this is achieved by feeding on better quality food (A/C ratios, 20% higher) and having lower maintenance costs (P/A ratio, 43% higher for aphids, therefore, respiration and nitrogen losses, 67% lower). This is presumably a reflection of a sedentary way of life (low respiration) and a specialized diet which requires little elimination of toxic nitrogenous material (low excretion).

Energy budgets and estimates of sap ingestion

Estimates of sap ingestion by phloem and xylem feeders is not possible by direct methods but insect energy budgets can give an indirect estimate which may be valuable in studying interaction between feeding insects and plant carbohydrate manufacture or where it is necessary to predict sap removal from plants. With aphid energetics influenced by so many biotic and abiotic factors any prediction or estimate can only be general unless much detailed information is also incorporated. Whilst this may be possible to organize, the relationships reported here are deliberately and necessarily simple because the detail is usually lacking. An easy task for the observer would be to count the number of adult aphids (large ones, with or without wings) and weigh a number of them to obtain the mean adult weight. In Figure 5-I I have related aphid adult wet weight to total energy consumption by that animal during its life time. There is a reasonable linear relationship for herbaceous
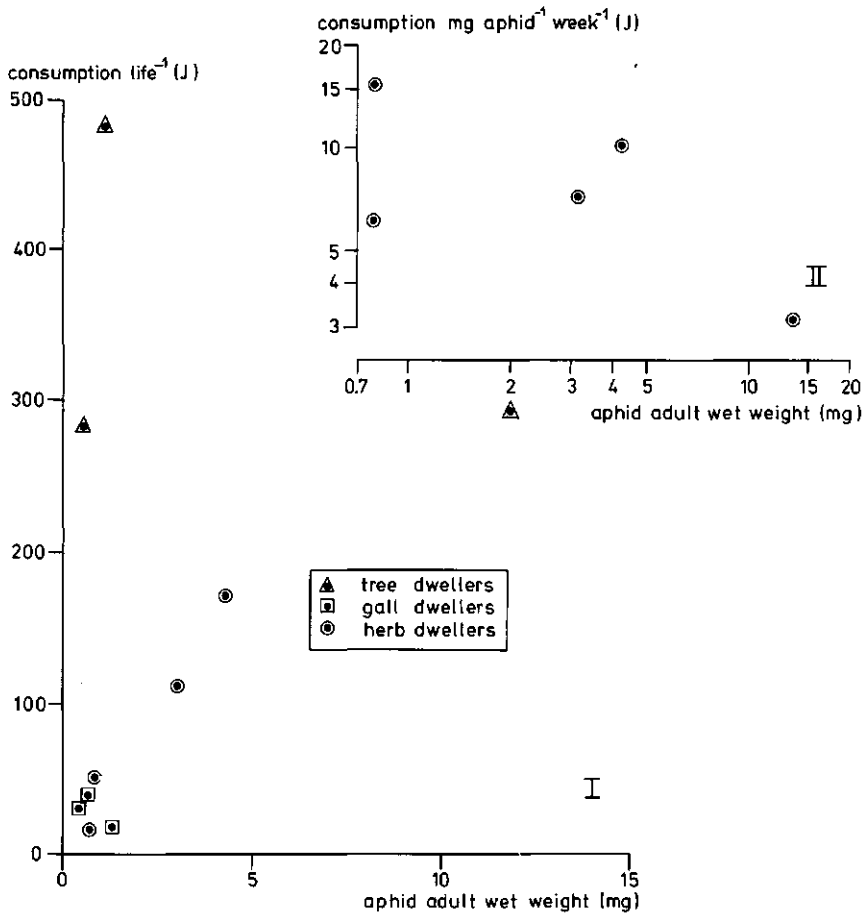
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Fig. 5. I: The relationship between adult wet weight and lifetime energy consumption. II: The relationship between adult wet weight and energy consumption.

plant feeders and tree seedling leaf feeders (both had similar A/C ratios and presumably similar food consumption). Tree feeding species, as might be expected, do not fit this pattern.

Thus, knowing the number of adult aphids and their mean wet weight, we can predict total energy consumption and this can be related to the volume of sap consumed, providing we know a little of its chemistry. Unfortunately aphid generations quickly become mixed in their age distribution and this relationship is of little predictive value. What is required is a relationship between adult weight and energy consumption per unit of aphid biomass and this is shown in Figure 5-II for the five aphid species with an A/C ratio greater than 50%. There is an inverse relationship, the larger the adult size the smaller the energy consumption in milligrams per biomass per week. With only five data points this relationship is of no practical use but we have the possibility of a very useful biological tool.
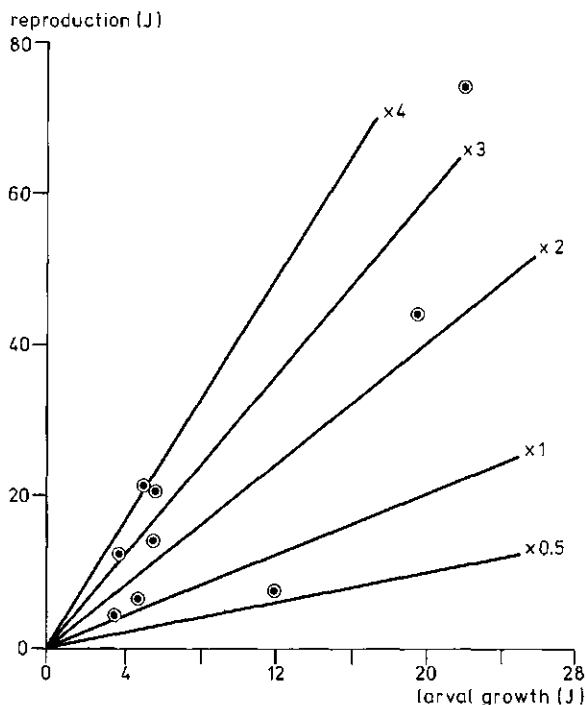
Fig. 6. Energy partitioning between growth and reproduction in aphids.

Energy partitioning between growth and reproduction in aphids

Recent studies on aphid reproduction have highlighted the complexity of the process and shown how fecundity may be related to adult weight attained which in turn may be partially related to the size of progeny. The energetics of growth and reproduction provide a way of summarizing the reproductive capacity of aphids and indicate the effectiveness of the trade-off between increase in body size and reproductive output. Figure 6 relates the energy content of larval growth to that of reproduction. The range of values is enormous with the lime aphid *E. tiliae* channelling only slightly more than half to its larval energy content into reproduction. *A. fabae,* on the other hand, channels four times its larval energy content into reproduction. There is no suggestion that the larger aphids are particularly poor reproducers.

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Toward a nutritional ecology of insects

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Abstract

A paradigm for a nutritional ecology of insects is presented, viewing nutritional physiology within the contexts of behaviour, ecology and evolution, according to the following logic. The favourability of natural environments is continually changing, placing constraints (e.g., low temperature and deterioration of food quality) on insects (and other organisms) that act to prevent them from attaining the physiological potential for their life history performance, resulting in loss of fitness. As a consequence, insects have evolved the ability to evaluate their environment and make decisions involving physiological, behavioural, and genetic responses that frequently have nutritional implications, affecting the consumption, utilization and allocation of food. The crux of these decisions (involving egg hatch, feeding, pupation, diapause, reproduction, etc.) consists of whether and when to alter, or to switch to, a particular response or set of responses. Research in nutritional ecology involves identifying how and why the decisions, responses and life history performance vary among species, populations and within a population through time.

Key-words: Spodoptera eridania, diapause, nutrition, consumption, growth

Most, if not all, aspects of an insect's existence, its physiology, behaviour, ecology and evolution, are strongly influenced by nutritional factors. For example, the amount, rate and quality of food consumed by a larva influences its performance i.e., growth rate, developmental time, final body weight, dispersal activity, and probability of survival (Scriber & Slansky, 1981; Slansky, 1982). In addition, performance by a larva will be carried over to affect subsequent performance by the adult (Slansky, 1982). Similarly, the amount, rate and quality of food consumed by an adult insect will affect its performance, timing and extent of reproduction, dispersal activity, probability of survival, and quality of offspring produced (de Wilde & de Loof, 1973a, b; Slansky, 1980a, b, 1982).

The evolution of the many different life styles exhibited by insects, varying in type of food eaten, extent of specialization in food, manner of feeding, degree of dispersal ability, length of life of the adult, which stage overwinters, etc., entails differences in how food is consumed, utilized and allocated (Calow, 1977; Slansky, 1980a, 1982; Townsend & Calow, 1981). Thus, a conceptual framework which links

physiology, behaviour, ecology and evolution within a nutritional context, a nutritional ecology of insects, will, by helping us structure and synthesize research on both the basic and applied aspects of insect life, allow us to achieve a better understanding of the evolution of different life styles (Slansky, 1982). Below, I outline the logic of the paradigm of nutritional ecology, and present examples to illustrate the major points of the paradigm. I conclude by discussing the application and value of a nutritional ecology of insects.

The paradigm of nutritional ecology

Life history performance and environmental constraints   Within an ideal, totally favourable environment (i.e., one consisting of an ad libitum level of food of high nutritional quality, an optimal temperature, humidity, etc., lacking predators, parasites and disease, and so forth) an individual insect will achieve its physiological potential in attaining the 'best' set of values for its life history performance. This particular combination of values results in the greatest reproductive contribution (i.e., fitness) possible for the particular genotype of that insect (Evans & Smith, 1952; Hairston et al., 1970). Clearly, however, the degree of favourability of natural environments is continually changing, with variations in the abundance and suitability of food, fluctuations in temperature and humidity, and appearance of predators and parasites being commonplace.

These changes in the environment act to reduce an insect's fitness by imposing constraints which prevent it from attaining the ideal values (as defined above) for its performance. For example, a drop in temperature may slow a larva's growth rate, resulting in prolonged development that increases its exposure to mortality agents and delays its metamorphosis to an adult. A deterioration in food quality may likewise slow growth rate, with similar consequences, and it may also result in a reduction in the larva's size at pupation, thus producing a smaller adult with decreased abilities to disperse and mate successfully, and with reduced fecundity. Because of natural selection imposed by these less than ideal environmental conditions, insects (and other organisms) have evolved the ability to evaluate their environment and make decisions involving physiological, behavioural and genetic responses. This ability allows insects to cope with environmental changes while attempting to achieve, or to alter, the ideal values for their life history performance (with various degrees of success).

Environmental evaluation, decision-making, and response   Insects undergo the process of evaluation, decision-making and response throughout their lives, and most of these decisions and responses affect, and are affected by, nutritional factors (Slansky, 1982). Perhaps one of the best known examples of environmental evaluation and decision-making by insects involves the induction of diapause. Numerous species are able to evaluate the photoperiod, and other features of the environment, and make a decision of whether to diapause or to continue development (Beck, 1980).

We can define decision-making generally as choosing between alternative courses of action (Dawkins & Dawkins, 1973; McFarland, 1977). One alternative is to con-

tinue to maintain physiological and behavioural processes in their current modes. For example, if the photoperiod is long enough, a larva continues its development rather than begin preparation for diapause, or, if a food presents suitable olfactory and gustatory stimuli, a larva continues to feed rather than search for different food.

Another alternative involves a compensatory response, whereby an insect attempts to achieve and maintain the ideal values for its performance in the face of a changing environment. It does this by altering its physiologial and behavioural activities, frequently by changing the level or rate of various processes. For example, many insects are able to reduce variation in their growth by considerably altering their food consumption and utilization in response to variation in the nutritional suitability of their food (Figure 1). The degree of compensation varies with insect species, type and magnitude of the change in food suitability, and other factors (Scriber & Slansky, 1981) and it is often not absolute (Figure 1).

It is one challenge of research in nutritional ecology to determine how and why different species differ in their compensatory abilities. This includes identifying how the 'costs' to fitness of a particular response compare with the costs of not making the response (Slansky, 1982; Townsend & Calow, 1981). For example, the costs to the response of increased feeding may include dilution of digestive enzymes and subsequent reduction in assimilation rate, and increased metabolic rate such that growth rate is not able to be maintained (Calow, 1977; Scriber & Slansky, 1981; Silby, 1981). A further cost may be an increased exposure to mortality agents (Slansky, 1982).

Some other examples of compensatory responses which are related to the consumption, utilization and allocation of food are: temperature regulation by increased basking and metabolic activity; water regulation by food consumption and by production of metabolic water; rejection of a courting male by a receptive female depending on its evaluation of the male's size and performance; and maintenance of egg quality through reduced egg production by adult females subjected to limited amounts or poor-quality food (Scriber & Slansky, 1981; Slansky, 1982).

A third alternative involves an inductory response, consisting of a genetically programmed change in the ideal values for life history performance. As mentioned previously, diapause induction is a classic example of an inductory response. Nutritional considerations related to diapause induction include a slowing of growth rate that prolongs development, allowing more food to be consumed, and associated changes such as an increase in lipid and glycerol synthesis (Scriber & Slansky, 1981; Slansky, 1982). Other inductory responses involving the consumption, utilization and allocation of food include the many cases of polyphenism (e.g. seasonal colour and size morphs, and alate vs. apterous forms; Shapiro, 1976) and the switch between reproduction and diapause/migration in adults (Rankin, 1978; Slansky, 1982).

The distinction between compensatory and inductory responses may at times be blurred, but regardless of how we categorize them, it is the central focus of nutritional ecology to identify the responses, their relationship to the consumption, utilization and allocation of food, and their consequences for fitness (i.e., their adaptive nature). In order to understand the evolution of these decisions and responses within their adaptive context, it is important to distinguish them from situations in
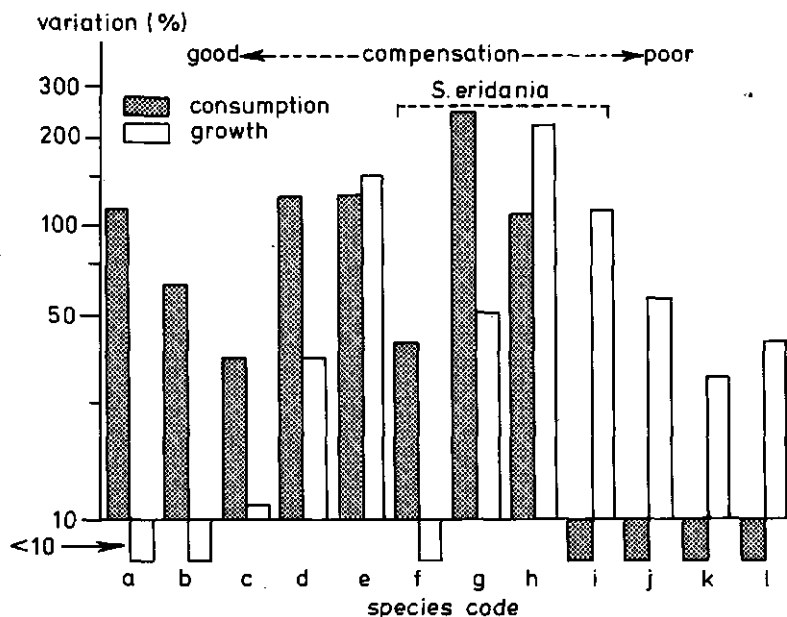
Fig. 1. Percentage variation in consumption and growth (calculated as [(largest-smallest value)/smalles value] × 100) for larvae of seven species of insects consuming various foods. Consumption is total dry weight consumed during the ultimate instar, and growth is fresh pupal weight, unless indicated otherwise. Note log scale on ordinate. a, *Plathypena scabra* on greenhouse vs. field grown *Glycine* leaves (Hammond et al., 1979); b, *Plutella xylostella* on 4 vs. 8 week old *Raphanus* leaves, growth is dry pupal weight (Taylor & Bardner, 1968); c, *Anticarsia gemmatalis* on young vs. senescent *Glycine* leaves, consumption is fresh weight, last 2 instars (Moscardi et al., 1981); d, same as b, on 4 vs. 8 week old *Brassica* leaves; e, *Epilachna varivestis* on leaves from 15 *Glycine* varieties (Barney & Rock, 1975); f, *Spodoptera eridania* on 4 vs. 20 week old cyanide-negative *Lotus* (Scriber, 1978); g, same as f on leaves from 10 *Medicago* varieties, consumption and growth are relative rates, penultimate instar (Scriber, 1979); h, same as f on low vs. high nitrogen-fertilized leaves from high-DIMBOA *Zea* variety, consumption and growth are relative rates, growth is dry weight (S. Manuwoto, unpublished data); i, same as h on low vs. high nitrogen-fertilized leaves from low-DIMBOA *Zea* variety; j, *Agrotis ipsilon* on artificial diet, 0 vs. 10^{-2} mol/l l-dopa, growth is dry weight, days 10 through 20 (Reese & Beck, 1976); k, same as j on artificial diet, 0 vs. 10^{-2} mol/l catechol; l, *Manduca sexta* on artificial diet, 0 vs. 2.5 mmol/l canavanine, consumption is relative rate, growth is maximum larval weight (Dahlman, 1977).

which a non-adaptive response is 'forced' on an insect by some feature of its environment. For example, the response of slower growth at a lower temperature is probably a non-adaptive, passive response forced on an insect because of its inability to fully compensate for the reduced temperature. The adaptive nature of changes in food consumption and certain other responses is often difficult to determine (Blau et al., 1978; Slansky, 1982).

The neurohormonal mechanisms underlying environmental evaluation, decision-making and response by insects are reasonably well known for decisions involving

feeding on a particular food, moulting, pupation, entering and breaking diapause, migration, and reproduction. On the other hand, the evaluation, decision-making and response interactions relating feeding, metabolism and growth to each other and to other behaviours are less well understood (Bernays & Simpson, 1982; Gilbert et al., 1980; Rankin, 1978; Scriber & Slansky, 1981; de Wilde & de Loof, 1973b; Slansky, 1982).

Application and value of nutritional ecology

Most of the decisions and responses that an insect makes during its life occur within a nutritional context. The responses may include changes in the amounts, rates and timing of feeding behaviour, metabolism, enzyme synthesis, food allocation, flight behaviour and other physiological and behavioural processes (Slansky, 1982). There are consequences for fitness, because of effects on survival and reproduction, resulting from these responses, and from the inability to achieve and maintain the ideal values for life history performance.

Thus, differences among populations and species in the kinds and magnitudes of their responses and in the ideal values for their life history performance are expected to have evolved as adaptations to different life styles. Herein lies the importance of the contribution of nutritional ecology to basic questions. By understanding the nutritional responses and consequences that occur throughout an organism's life, we will achieve an understanding of the ecology and evolution of different life styles expressed as species that are migratory or non-migratory, specialized or generalized feeders, herb or tree feeders, parasitic or predatory, and so forth.

The fact that this basic information also has applied relevance to the development of integrated pest management strategies has only recently been recognized (Barfield & Stimac, 1980; Levins & Wilson, 1980). For example, we must know the factors influencing the feeding, growth, reproduction, dispersal and survival of pest insects if we are to refine and couple models of pest population dynamics with models of crop plant growth. This will produce models with greater precision and greater powers of prediction of pest population size and crop plant damage (Barfield & Stimac, 1980; Stimac, 1982). This information may also allow us to effectively manipulate the crop environment (such as through chemical fertilization and growing of resistant plant varieties) to disrupt the normal performance of insect pests (Jones, 1976; Norris & Kogan, 1980; Tingey & Singh, 1980). Thus, structuring and synthesizing research on insects within the paradigm of nutritional ecology will yield much useful information of broad relevance.

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Gypsy moth (Lymantria dispar): induced feeding preferences as a bioassay for phenetic similarity among host plants

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Abstract

A new experimental method, designed to elucidate patterns of host plant similarity as perceived by feeding lepidopterous larvae, is proposed. Gypsy moth larvae easily discriminated between black cherry and three fagaceous species (black oak, white oak and beech), but apparently confused the latter three species. Perceived similarities among these plants were not only a function of plant taxonomic affinity, but also of previous larval experience. Larvae reared on cherry ate less cherry foliage in feeding tests (learned aversion) and discriminated among the fagaceous species more finely than did caterpillars fed on the oaks or beech. These patterns were probably due to age-specific or host-induced changes in food acceptance, rather than differential selection in treatments reared on alternate hosts. This method is suggested as a means of identifying distinctly perceived pairs of hosts for use in agricultural pest control, and to elucidate whether plant-specific factors govern patterns of plant similarity to insects.

Key-words: Lymantria dispar, food preferences, aversion learning

In this paper, I describe experimental methods clarifying phenetic similarities among potential hosts of an insect population. Evolutionary history and present-day design of an insect necessarily constrain the degree of host discrimination displayed by the sensory system (Dethier, 1970, 1980; Chapman & Blaney, 1979), physiology (Gould, 1979) and behaviour (Waldbauer & Fraenkel, 1961; Waldbauer, 1962, 1964). The above studies indicate the existence of phenetic similarities between distantly related groups of plants when filtered through (or perceived by) the sensory receptors or physiology of an individual insect. If the ordering or clustering of potential hosts by insects were known, it would be possible to predict the likelihood of certain future changes in host range (Gould, 1979).

Lepidopterous larvae show ontogenetic changes in feeding preferences: prior feeding on a host induces (increases relative) preference for that species (Stride &

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Straatman, 1962; Jermy et al., 1968; Hanson, 1976). The gypsy moth *Lymantria dispar* L. is no exception to this rule (Barbosa et al., 1979). The phenomenon of induction is used here as a bioassay of how larvae perceive their hosts, for just as insects that have been reared on the same host show similar feeding preferences, insects reared on hosts that present similar physical or chemical profiles should show homogeneous behaviours in the test arena. In accordance with this conceptual framework, I tested the feeding preferences of larvae previously fed on four tree species, and examined the insect-perceived similarity or difference of those species as measured by behavioural patterns of larvae reared on them.

Materials and methods

Newly hatched gypsy moth larvae (source: Northport, New York) were placed on foliage of black cherry *Prunus serotina*, black oak *Quercus velutina*, white oak *Quercus alba*, and beech *Fagus grandifolia*. The petiole of each spring was placed in a florist's aquapic punched through the side of a 250-millilitre paper cup (about 25 larvae per cup). Foliage was replaced every second day; water was renewed ad lib. Beech bud scales were removed during the first days of rearing.

Feeding preferences were tested in arenas modified from Jermy et al. (1968). Larvae were placed in a plastic 10-centimetre Petri dish half lined with moistened filter paper, with three 1-centimetre leaf discs from each of two of the host species. Leaf discs were arranged in an ABABAB fashion, so that larvae showing preference for one host would encounter an alternate disc type before termination of the experiment (Jermy et al., 1968). Feeding experiments were ended when approximately 50% of one species' leaf area had been consumed. Larvae were tested once and discarded. Feeding preferences of larvae reared on each plant were tested for all possible pairs of plant species. Larvae were tested within an 8-day period because previous work (Wasserman, unpubl.) indicated that tree phenology drastically affects relative acceptability of these hosts. Hence larvae of different histories were tested at alternate physiological ages: Instars 4 and 5 for larvae from oaks and beech, and Instars 3 and 4 from cherry. At least ten replicates were run for each inducing host and test plant pair combination.

Preference data were transformed to arcsines, to approximate normality, before statistical analysis. To test the overall feeding preferences of larvae derived from each host, I performed a multivariate cluster analysis, with inducing plants as operational taxonomic units (OTU's) and means as characters. Cluster analyses based on means and standard deviations yielded qualitatively similar results and will not be reported here. Characters were normalized, similarities among OTU's computed as product-moment correlation coefficients and clustering was achieved by an unweighted single-linkage method.

Results

Feeding preferences of larvae from each inducing plant are compiled in Table 1. No group preferred cherry in any feeding test: only 6 of 174 larvae ate more cherry foliage than foliage of the alternate host. The qualitative host preference of larvae

Table 1. Feeding preferences of gypsy moth larvae previously reared on one of four tree species. Preference in an A : B test arena is defined as percentage of species A leaf area eaten/percentage of total leaf area eaten.

Inducing host →	Cherry	Beech	White oak	Black oak
Black cherry : beech				
Y	0.0524	0.1452	0.1548	0.0883
S.E.	0.0301	0.0489	0.0525	0.0299
n	14	15	15	15
Black cherry : white oak				
Y	0.0391	0.0587	0.2609	0.0407
S.E.	0.0215	0.0219	0.0723	0.0224
n	15	14	10	17
Black cherry : black oak				
Y	0	0.1790	0.1116	0.1303
S.E.	0	0.0350	0.0303	0.0355
n	12	19	21	17
Beech : white oak				
Y	0.6232	0.5490	0.4103	0.4547
S.E.	0.0956	0.0557	0.0457	0.0678
n	12	23	22	15
Beech : black oak				
Y	0.3902	0.4779	0.4076	0.4547
S.E.	0.0637	0.0737	0.0471	0.0471
n	15	19	15	18
White oak : black oak				
Y	0.5389	0.4916	0.4580	0.4705
S.E.	0.0983	0.0433	0.0450	0.0404
n	10	24	12	18

depended upon previous experience: individuals reared on oaks showed the preference hierarchy: black oak > white oak > beech > > black cherry; those reared on beech: black oak > beech > white oak > > black cherry; cherry-reared larvae the non-transitive preference hierarchy: (black oak > beech > white oak > black oak) > > black cherry. Ordering of preferences within the fagaceous species was never significant by sign test (12 two-tailed tests, $P \geqslant 0.118$).

In only one of six pairwise comparisons was there evidence of induction of feeding preference: the comparison black oak : white oak ($t_s = 1.799$, 43 df, $P = 0.0395$, one-tailed). The reverse relationship appears in comparisons including cherry. Cherry-reared larvae ate significantly less cherry than larvae from host X for all three black cherry: X comparisons.

Analyses clustering host plants by the behaviours of larvae fed on them are pre-
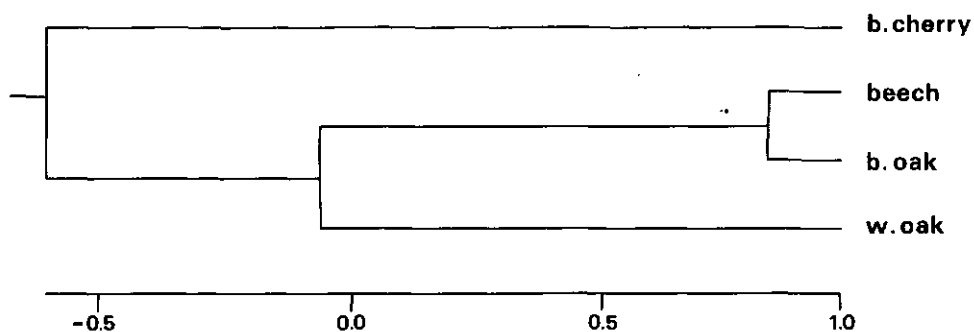
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Fig. 1. Phenograms from cluster analysis of feeding preference. Mean preference of larvae of various feeding histories used as characters to indicate similarity among 'inducing hosts'. Pairs of species are more tightly clustered (yield behaviourally similar larvae) toward the right.

sented in Figure 1. Composite feeding preference differed greatly between cherry- and Fagaceae-reared larvae. Within the Fagaceae, larvae derived from black oak and beech were clustered behaviourally, with slightly divergent preferences seen in white oak-reared individuals. The underlying explanation for this phenogram can be understood by subjecting these data to principal components analyses, a means of reducing high (6) dimensionality of these data to a few interpretable axes or 'components'. The first two principal components explain over 96% of the variation. When 'inducing plant species' are projected onto these axes (Figure 2), it is seen that Factor (principal components axis) 1 is a general 'cherry vs. non-cherry' axis. The factor is heavily influenced by the comparisons black cherry : beech, black cherry : black oak, beech : white oak and white oak : black oak. The second factor, separating white oak from the cluster beech/black oak, is highly influenced by black cherry : white oak and beech : black oak comparisons.

Discussion

The induction of feeding preference, demonstrated in several Lepidoptera (Jermy et al., 1968; Ting, 1970; Yamamoto, 1974; Hanson, 1976; Greenblatt et al., 1978; Barbosa et al., 1979; Chew, 1980), has been interpreted (Jermy et al., 1968; Hanson, 1976) as an insect species-specific trait; generalized feeders might show a stronger modification of preference than more restricted feeders. Ting (1970), however, suggested that the absence of induction is plant-specific: certain hosts are unable to alter the preference of that insect. I propose that 'strength of induction' reflects the degree to which two hosts are sensorially distinguished by the insect. If two test hosts are phenetically distinct to the larvae, induction will appear strong: weak or no induction implies confusion of the larvae by the hosts. Congeneric species, sharing a number of physical and chemical properties, should be phenetically more similar than more distantly related species. The lack of demonstrable induction among
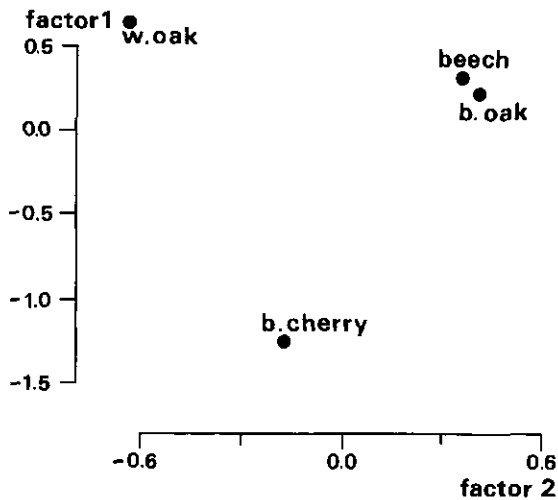
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Fig. 2. Principal components analysis of feeding preference experiment. Mean preference of larvae of various feeding histories were used as characters to indicate similarity among 'inducing hosts'. Factors 1 and 2 represent linear combinations of characters along axes of greatest variation among inducing hosts.

fagaceous species might be caused partly by the modest sample sizes used here, but also by confusion of oak species by feeding gypsy moth larvae. Independent support for the latter contention would be the observation that first instar larvae show stronger preference in comparisons of distantly related hosts than closely related ones. Barbosa et al. (1979) presented first instar gypsy moth larvae with leaf discs of pairs of potential hosts. Larvae offered pairs of fagaceous species, as predicted, showed more homogeneous feeding preferences than did larvae presented with a fagaceous/non-fagaceous pairing (one-tailed Mann-Whitney test on 'difference in leaf area consumed from either species/total leaf area consumed': $U = 16$; $n = 3$, 6; $P = 0.05$). Of course, larvae are not totally confused by confamilial hosts: larvae herein and in Barbosa et al. (1979) induced preferences within the Fagaceae.

The perceived difference between two host species is not only a function of their degree of relatedness or chance phenetic resemblance, but also depends upon prior experience of the larva. I tested whether the three fagaceous species appeared more homogeneous to cherry-reared than beech- or oak-reared larvae, by calculating the variance among arcsine-transformed mean preferences in the comparisons beech : white oak, beech : black oak and white oak : black oak. If all fagaceous species were perceived similarly, this value would be near zero. (Induction of preference would artificially raise the variance for larvae derived from fagaceous species.) All three F-ratios are in the same direction (variance among means of comparisons from cherry-reared individuals greater than for Fagaceae-reared larvae); one comparison is highly significant ($P = 0.004$), another of borderline significance ($P = 0.054$).

The inability of Fagaceae-reared larvae to distinguish among fagaceous hosts

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might be a result of selection (differential mortality due to inducing plants) or of ontogenetic processes. For selection to create this pattern, larvae on beech or oaks must exhibit greater selective mortality, leading to a narrowing of preferences, than their cherry-reared counterparts. However, mortality was lowest in oak and beech cups. Second, it is possible that as gypsy moth larvae mature they naturally become more tolerant of novel hosts: the cherry-reared group was tested at the lowest physiological age. Field and experimental observations that bear on this phenomenon (e.g., Barbosa et al., 1979; Wasserman & Schlichting, unpubl.) are usually confounded by the co-occurrence of plant phenological changes; hence, this possibility will not be evaluated here. Third, it is possible that receptor sensitivity is affected differentially by cherry and other hosts. If fagaceous hosts contain chemicals lowering receptor sensitivity to differences among plants in general, then larvae derived from these plants will more likely accept any host, including cherry. This mechanism would also explain the apparent 'reverse induction' (or learned aversion) caused by feeding on cherry leaves, i.e., that larvae from cherry ate less cherry than did other larvae, when cherry leaf discs were presented in the test arena (six of nine comparisons significant at $P \leqslant 0.063$). The validity of this hypothesis could be tested by asking whether other potential hosts are more acceptable to oak- or beech-reared larvae than to larvae from cherry.

The methodology described here is a behavioural assay of insect-perceived patterns of host affinity, which might elucidate future patterns of host utilization by these insects. Feeding preference alone does not govern patterns of host utilization. Therefore, to predict the probability of utilization of a novel host by gypsy moth, one must consider results of similar studies of adult behaviour and physiological tolerance of feeding stages, in conjunction with studies of feeding behaviour. The predictions evolved by such analyses can be applied to two problems. First, they can identify pairs of plants that are perceived by pest species as drastically different. If these plants are economically important, they might be planted in successive seasons or interplanted within single fields, lowering the rate of adaptation by the herbivore to either. Second, parallel assays of the same hosts could be run using several insect species, to discover whether patterns of host similarity are repeated among insect taxa. From this we might gain insight into the degree to which these patterns are plant specific or a function of the evolutionary history of each insect species.

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Studies on insect-plant relations in China: an overview

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Abstract

Studies on insect plant relations in China are overviewed. The paper is limited to host plant relations of traditional beneficial insects, insect pests in agriculture and forestry, and the physiological effects of secondary plant substances on both beneficial and noxious insects.

Key-words: Bombyx mori, Antheraea pernyi, Meromyza saltatrix, Nilaparvata lugens, Pyralidae, *Dendrolimus* species, secondary plant metabolites, ecdysones, pest control, food preferences

The knowledge concerning the relations between insects and their host plants is subject to study by disciplines in the broad fields of agriculture and forestry. It touches on the rearing and multiplication of beneficial insects such as silkworms, the science of plant protection, and the use of secondary plant substances to increase the production of useful insect products and to kill or sterilize pest insects. The hearsays and exact scientific information relevant to this problem in China can be regarded as a large stock of entomological knowledge which, when properly collected, systematized and organized, could give rise to extensive handbooks. Of course the discussion of all these items needs space and I am not allowed to do so here. I would rather like to take this opportunity to make a brief introduction about the related conditions in China in the hope that you may understand something more about China as she has not sent participants to attend the previous several symposia of this series. I will limit my paper to the following three headings: firstly, on host plant relations of some of our traditional beneficial insects; secondly, on some important insect pests in agriculture and forestry; and thirdly, on physiological effects of some secondary plant substances on some beneficial as well as injurious insects.

Host plant relations of traditional Chinese beneficial insects

The mulberry silkworm Bombyx mori   At present it is believed that *Bombyx mori* is

derived from *Bombyx (Theophila) mandarina*. The haploid chromosome number of *B. mori* is 28, of *B. mandarina* 27. Kawaguchi (1928) observed that one of the 27 chromosomes of *B. mandarina* pairs with two chromosomes of *B. mori* at maturation division of F_1 hybrids. He therefore assumed that one of *B. mandarina* chromosomes might have split into two during the history of domestication. But *B. mandarina* also has a race with 28 chromosomes.

Nearly five thousand years ago, the central part of China was covered with many mulberry groves and forests with *Bombyx mandarina* feeding on the leaves. The host plants of *Bombyx mandarina*, and later of *Bombyx mori*, are some species of the genus *Morus*, of which *Morus alba* is the most important. It has several varieties or subspecies which can be used for rearing the then domesticated *Bombyx mori*. This insect is strict in its food habit and may be regarded as monophagous; but it can eat *Cutrania* in addition to *Morus* in the Moraceae. An old Chinese book of the sixth century records that the leaves of *Cutrania* can be used to rear the silkworm to produce silk good for manufacturing music instruments with strings of high quality. They will produce better sounds than the strings made from the silk of the silkworms eating leaves of *Morus alba*.

Some strains of the silkworm can take up carotenoids from the host plant leaves, let them go into the silk glands in the middle of the last (fifth) instar and spin yellow cocoons. Yellow cocoon was first recorded in the books of Han dynasty; therefore, the strains producing yellow cocoons probably first arose about two thousand years ago.

Silkworm diseases are the main threat to the enterprise of silkworm rearing in China. In the sixties it was found that diseases of silkworms are intimately related to the quality of the mulberry leaves on which the larvae feed. The people in some regions of South China used to cultivate mulberry groves into areas famous for their high yield of the leaves. But these leaves are soft tender, and we usually call them the 'immature' leaves. The silkworms eating these leaves will have a rate of infection by pathogens varying from 30% to 75%. Chemical analysis of these leaves showed that they contain excessive amounts of organic acids, especially oxalic and fumaric acid. The latter can serve as an indication of foliage 'immaturity'. In addition to these, tartaric acid, citric acid and succinic acid are also present. Mature leaves have a larger amount of succinic acid and small amounts of oxalic acid. The larvae feeding on 'immature' leaves will excrete more oxalic acid with their faeces in the early stage of the fifth instar, but this capacity seems to fade away at a later stage in this instar. As a consequence the acid will accumulate in the digestive tract and weaken the larvae. They may then contract diseases. At the same time the high water content of the 'immature' leaves also plays a negative role in affecting the condition of the later instars though not so in the early instars.

The oak silkworm Antheraea pernyi This silkworm is not reared indoors. In rearing this silkworm one has to provide the right kind of host plants and grow them in well managed groves on hills. It feeds on the leaves of the trees belonging to the genus *Quercus* (Fagaceae), but also can eat the leaves of *Salix viminalis* (Salicaceae). We do not know what chemicals are responsible for the feeding preference, but tannins seem to be important and the cocoons are so heavily tanned that silk

reeling is difficult.

Experiments have been carried out in Liaoning Province to ascertain which species of trees are most suitable for oak silkworm rearing and it was found that *Q. liatungensis* would give the best result, followed in descending order by *S. viminalis*, *Q. acutissima*, *Q. mongolica*, *Q. dentata* and *Q. aliena*. Spraying dilute solutions of some salts such as $MnSO_4$, $CuSO_4$, $CoSO_4$ on the leaves can promote larval growth and prevent pathogenesis to a certain degree.

Gallnut aphids, the white way scale and the lac insect This group of Homoptera includes *Schlechtendalia chinensis*, *Nurudea* spp., *Ericerus pela*, and *Laccifer lacca*. Gallnuts, insect wax and lac are useful materials which had been used in medicine and for other purposes more than one thousand years in China. Within the last thirty years the Chinese entomologists paid lots of attention to increase their production and met many problems related to host plant relations. For example, in order to improve artificial multiplication of the gallnut aphid *S. chinensis* we have to pay equal attention to the aphid, the primary host i.e., the sumach plant, and the secondary host i.e., the moss *Mnium*, which constitutes the three essential requisites for the production of gallnuts.

Host plant relations of agricultural and forest pest insects

This is a very large area from which I choose to mention something about the rice borers, the brown rice planthopper, the wheat stem maggot, and the pine caterpillars.

Rice borers Rice is the staple grain of China. The important rice borers belong to the family Pyralidae. They are the yellow borer *Tryporyza incertulas* and the striped borer *Chilo suppressalis*. The noctuid *Sesamia inferens* or the pink borer may cause serious damages in the rice fields in some districts but it is not so important as the former two species.

The phenological relations between the rice borers and the rice crops had attracted the attention of Chinese entomologists early in the twenties. The egg masses are laid on the rice plants, but only a small portion (about 5-7%) of the newly-hatched larvae can bore into and establish themselves within the rice plants. Whether they will be successful in doing so is largely determined by the growth stage of the rice plants. For several decades we paid much attention to adjust the times of sowing the seeds and transplanting the seedlings to avoid the synchronization of the vulnerable growth stages of the rice plants such as tillering and heading with the peak of oviposition by the borer moths. The borers will produce 'dead hearts' in the tillering stage and 'white heads' in the earing stage. Between tillering and earing there are two other growth stages which are called the rounding up and node lengthening. Probably due to the thickening of the sclerenchymatous tissues in the rounding up stage the rate of successful boring into the rice plants is only about 5-6%, but in the earing stage it reaches 34%, in the heading stage it is 23% and in the milking to ripening stage it declines to 9%. The borers will cause heavy damage when the peak of oviposition by the moths coincides with the tillering or earing of
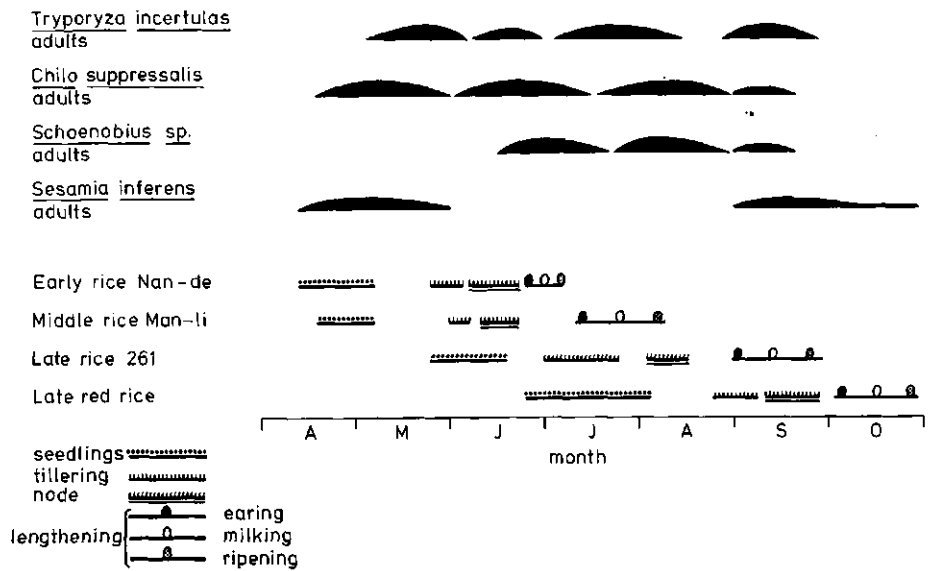
Fig. 1. The relationship between rice borers and rice crops (Chang-sha, 1957).

the rice plants. During tillering the plants have a deep green colour which will attract the moths for oviposition. The complex relation between the rice borers and the rice crops is illustrated in Figure 1.

It is interesting to note that the growth of the larvae within the rice plants is influenced by the watering condition of the rice fields. Recently we have found that alternative drainage and watering of the rice fields can bring forth a conspicuous reduction of borer infestation and increase of rice yield. This is caused by hindrance of the penetration by the borers, adverse effect on larval growth and increasing the tillering capacity of the injured rice plants.

The brown rice planthopper   In recent years the brown planthopper *Nilaparvata lugens* has become a serious pest of the well fertilized rice fields; but some rice varieties are resistant to it. It is found that the resistance is due to the lack or low quantites of the free amino-acids aspartic acid, asparagine and valine which are said to be essential for the planthopper to keep on feeding.

The wheat stem maggot   The wheat stem maggot *Meromyza saltatrix* is the chief insect pest in the spring wheat belt in North China. The degree of infestation is obviously different among different spring wheat varieties. The adult shows strict oviposition preference. The resistant wheat varieties are characterized by early maturing, especially the fast growth in the early stages, the narrow shape of the leaf bases, and the pilosity of the leaves.

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The pine caterpillars They are the most destructive insects of our pine forests. They belong to the Lasiocampidae and are specific in their host plants. The following six species cause serious damages in our forestry: *Dendrolimus suprens* (Butler) (= *D. sibiricus* Tsch.), distributed in the north-eastern provinces and the northern part of Sinkiang; *D. tabulaeformis* Tsai & Liu in North China; *D. spectabilis* Butler in the provinces around the gulf of Pohai; *D. punctatus* (Walker) in South China; *D. latipennis* Walker in Yunnan Province; and *D. kikuchii* Matsumura in South China. Each species has its own preferred host trees, e.g. *D. punctatus* prefers *Pinus massoniana*; *D. spectabilis* prefers *P. densiflora,* and so on. The chemical nature of the secondary substances in the pine needles responsible for this difference are not known, but is is interesting to investigate them. Moreover, the work in the sixties has shown that *D. spectabilis, D. tabulaeformis,* and *D. punctatus* could be easily inter-crossed. The eggs laid after the crossing of *D. punctatus* and *D. tabulaeformis* could have hatchability rates higher than 90%; and the progeny of the first generation could grow and develop normally. The progeny of the first generation from the crossing of *D. spectabilis* and *D. tabulaeformis* could develop into male and female moths with a certain capacity of egg-laying. These facts indicate that they are recently differentiated into separate species with their own preferred host plants.

The physiological activities of secondary plant substances towards insects

Plant toxicants or insecticides The utilization of certain plant materials as insecticides has a long history in China. In 1958 we had a mass movement to investigate and apply the so called 'indigenous insecticides' for killing medical and agricultural insect pests. It was found that the following plants were really good for this purpose: *Agave americana* (Amaryllidaceae), *Camellia sasanqua* (Theaceae), *Melia azedarach* (Meliaceae), *Moghania prostrata* (Papilionaceae), *Rhododendron molle* (Ericaceae), *Stemona japonica* (Stemonaceae), and *Veratum nigrum* (Liliaceae).

Sterilizing plant compounds Several years ago laboratory experiments were carried out to use the alkaloid camptothecine from *Camptotheca ecuminata* and harringtonine from *Cephalotaxus fortunei* to carry out sterilization of *Dendrolimus punctatus*. Contact tests were made by mixing the alkaloid with lanolin (using acetone as solvent) and smearing the mixture on the cage screen. Caterpillars of fifth and sixth instars which had contacts with the alkaloid would develop into moths laying eggs with very low hatchability. Spraying the alkaloid in acetone (0.05 and 1 g/l) to newly emerged moths would cause them to lay sterile eggs (92% and 100% respectively). It could also inhibit the embryonic development when applied to the eggs. But harringtonine is less effective than camptothecine.

Phytoecdysones for silkworm rearing In the seventies we started to use juvenile hormone analogues (JHA) to increase silk production of *Bombyx mori.* This is done by spraying the emulsified JHA to the mulberry leaves and feeding the fifth instar larvae 72 h after the fourth moult with these leaves or spraying it directly on the body of the larvae. The instar is then prolonged to 1 - 1.5 more days. The cocoons will be larger and contain about 10% more silk. But the larvae would start to spin

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cocoons quite irregularly in time and thus more labour is needed to take care of them. We looked for phytoecdysones from many plants and used them through oral administration at the later stage of larval development to make the time of cocoon formation uniform. It was found that many plants including *Lamium barbatum* (Labiatae) can serve as sources of phytoecdysones. The β-ecdysone in *L. barbatum* amounts to 0.9 g/kg of the dry matter of the whole plant.

Conclusion

The contents under the three headings I have reported perhaps may give some idea about the variation of the knowledge concerning the relations between insects and plants needed in China. As you know, China is a vast country with rich flora and fauna; therefore more intensive studies on the relations between insects and plants are needed. We look forward to more contacts and exchange of views with our colleagues from other countries to explore this area of scientific research.

Ecological aspects of insect-plant relationships — round-table discussion

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Insect ecologists are concerned first of all with establishing and understanding patterns of distribution and abundance of insects in ecological time. What factors affect the natality and mortality of natural populations? How do these factors interact to produce the observed patterns? How and why do such patterns alter in response to natural and man-made perturbations? At the same time, insect ecologists are concerned with longer-term changes through evolutionary time. What kinds of evolutionary responses have been brought about in insects as a consequence of the selective action of different mortality factors and how is the direction of evolution influenced by different ecological circumstances? To what extent is the evolution of an insect population restricted or catalysed by its past evolutionary history?

Like other branches of science, ecology will advance rapidly only if we attempt to generalise from a variety of well-understood, specific examples or case histories. The judicious construction of hypotheses or models helps to guide future research that is designed to test and attempt to falsify such hypotheses. This will permit us, eventually, to determine the extent to which natural patterns are predictable in terms of underlying ecological and evolutionary processes.

The topics included in this discussion represent rather well the different levels of approach being taken in research on the role of host plants in shaping the ecology and evolution of phytophagous insects. They range from detailed studies of particular insects to more general and even speculative models of ecological interactions. They address the two major aspects of an insect's relationship to its food plants: first, location and recognition of host plants, and second, growth and survival on the host plants once they are found.

Host-finding behaviour

Finch introduced this subject by summarising some recent studies showing the

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importance of anemotaxis in searching behaviour by the cabbage root fly, *Delia radicum* (L.). This fly is widespread throughout temperate regions of the northern hemisphere, where it specialises in attacking plants of the family Cruciferae (Brassicaceae). The larvae mine the roots of developing plants and the insect is a serious pest of *Brassica* crops. Since cruciferous plants, even in agricultural plantings, are relatively ephemeral and patchy in distribution, the female flies must be highly mobile and must be readily capable of finding new patches of host plants on which to lay their eggs. It has long been known that allylisothiocyanate (ANCS), a volatile mustard oil released from cruciferous plants, is attractive to the female flies but the distance over which this compound is effective and its role relative to other aspects of host-finding behaviour have remained unclear. There seem to be three hypotheses. First, flies move randomly until encountering the odour of ANCS at a short distance from the host plant (cf. Thorsteinson, 1960). Second, flies exhibit positive anemotaxis, flying upwind whenever they encounter the host odour, even at considerable distances from the odour source (cf. Kennedy, 1965). Third, the females fly upwind, or at some angle to the wind, until they encounter the host odour in the immediate vicinity of the host plants.

Finch described an elegant series of field experiments undertaken by himself and Skinner in central England during 1978 and 1979 to distinguish between these three hypotheses. Two large grass arenas were prepared, each 100 m in diameter. The first was continuously exposed to host plant odour, provided by a 10-metre wide band of a *Brassica* crop planted around the periphery. The second arena was surrounded by a 10-metre wide band of mowing grass, *Lolium perenne*, and served as a control. In a series of 18 experiments in different wind conditions, a total of 72 000 laboratory-reared female flies were released at the centres of the two arenas. Their subsequent flight directions were then monitored by a series of yellow traps spaced evenly around the peripheral *Brassica* and *Lolium* plantings. The traps contained ANCS in one series of experiments and water in another, thus providing four treatments altogether, ranging from a 'double dose' of host odour (*Brassica* arena + ANCS traps) to no host odour (*Lolium* arena + water traps) blowing downwind over the release point.

The recapture results were clear-cut. Analysis by a method based on circular variance showed that when the wind run was less than 200 km/day flies were caught most frequently in the upwind sectors of both arenas, regardless of wind direction or of whether the traps were baited with ANCS or water. When the wind run exceeded 200 km/day, most females were recaptured in the two sectors adjacent to the upwind sector of each arena, presumably because they were unable to fly directly upwind. The investigators were therefore able to conclude that females ready to lay eggs fly upwind naturally, without having to be stimulated by odours from a host crop. The role of host odours, including ANCS, seems to be restricted to host selection over short distances (up to circa 5 m). In the case of *D. radicum*, therefore, the first two of the initial hypotheses concerning host location can be rejected in favour of the third.

Replying to a question by Jepson, Finch indicated that vision is important in host searching by the cabbage root flies but that it only operates over distances of about 2.5 m. Surrounding the experimental arena with a more heterogeneous group of

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Brassica plants, containing larger and visually more attractive plants, would not have caused the flies to diverge from their upwind flight. He agreed that the distance over which host odours are attractive has probably been exaggerated in the past; present evidence indicates that it extends only to about 5 m or, at most, 10 m. Prokopy asked if the results could have represented the responses of flies that were in an 'escape mood' as a result of experimental manipulation. In reply, Finch pointed out that steps were taken to minimise such an effect; the flies were released in the arenas the previous night and thus had several hours during which to warm up and walk around in the grass the next morning before taking flight. To minimise the effect still further, by conducting experiments lasting longer than one day, was impossible because the frequent changes in wind direction would have obscured the results.

Thibout wondered why research on insect attraction has concentrated on the relatively stable end products of enzyme action on precursor compounds in plants whereas relatively unstable intermediary compounds or side products have been all but ignored. Some of these intermediate compounds do seem to be important and by their very lability could provide more useful information to insects. Finch replied that he used ANCS for most of his research because, though not the best isothiocyanate, it is effective and inexpensive. Although other crucifer compounds are attractive to insects, he had found that traps baited with ANCS were as effective as traps baited with more complex extracts of various crucifers. This suggests, for his crucifer-insect system at least, that a given fraction of the insect population is responsive at any one time and that which particular attractant mixture is used does not greatly affect the trapping results.

Stockel next discussed the annual movements of females of the European corn borer, *Ostrinia nubilalis*, from non-host plants onto maize, the larval host. In France, where Stockel and coworkers conducted their experiments, the adult moths generally appear during May and June in and around fields of cereal crops such as wheat, barley and oats. Though not larval hosts, these crops apparently serve as 'rendez-vous plants' for the two sexes prior to infestation of maize fields during July. Elucidation of this phenomenon is not only of general ecological interest, but also vital for appropriate application of mating disruption techniques to the control of this major insect pest.

Starting in mid-June, Stockel released laboratory-reared corn borers in two adjacent fields, each about one hectare in area. Insects released in the first field, consisting of wheat, were coated with a red fluorescent dust wheareas insects released in the second field, consisting of maize, were coloured green. Light traps, placed in both fields during the month of July, revealed that the released moths were moving only from the wheat field to the maize field; no movement was recorded in the reverse direction. Total trap catches of both sexes were greater in the maize than in the wheat (167 versus 55 for females; 214 versus 172 for males), indicating that the population density of the insects was highest in the maize field.

In contrast to the light-trapping results, traps baited with synthetic sex pheromone caught substantially more male moths in the wheat field than in the maize during the same period. Stockel suggested two likely explanations for these results. First, males display a greater tendency to remain in the wheat field (the 'rendez-

vous' or 'refuge' plant) than do females. Second, sex-trapping results are profoundly influenced by competition with wild females: fewer males are caught in the maize field because of the greater availability of receptive females, whereas males are more readily captured in the wheat field because of the relative scarcity of females. In either case, there are profound implications for using sex traps in pest control. Pheromone application must be started early, before the moths move into the maize fields, and trapping must extend beyond the maize fields themselves in order to reduce the influx of mated females from adjacent areas.

In the third contribution to the discussion of host-finding by phytophagous insects, Hawkes outlined a simple simulation model that he had developed to explore the possible influence on host-finding success of variation in patterns of host-plant dispersion and of the degree of directionality exhibited by the searching insects. The model was designed to simulate host-searching by adult insects of species that attack relatively ephemeral plants whose distribution is patchy in space and time. In the model arena, the area occupied by host-plant 'habitat' was kept constant at 1% of the total area but its pattern of dispersion was varied. For each simulation, hypothetical insects were considered to make 1000 short straight movements of standard length within the conceptual arena and success rate was recorded simply as the number of 'insects' encountering host plants. By altering the programmed relationships between successive movements, different degrees of directionality in insect searching could be simulated for any given pattern of host plant dispersion.

In the first set of simulations described by Hawkes, the distributions of plant patches were considered to be independent of those in the previous generation. The 'insects', in other words, had to start afresh each 'season' without prior 'experience' in the arena. Under these conditions, aggregated plants were invariably encountered less frequently than were randomly or regularly distributed plants. This result was obtained regardless of the degree of directionality used by the searching insects, though success rate in both regularly dispersed and aggregated environments increased with increasing degrees of directionality. Thus highest success rates were obtained with straight flights and regularly distributed habitats.

Hawkes next described the outcomes of simulations in which the distribution and predictability of aggregated host plants are more stable from each insect generation to the next — probably a more realistic scenario for most insects associated with ephemeral plants. Under these conditions, random movement was found to be superior to all other levels of directionality. Although simulations revealed progressively lower success rates as movement patterns became less random, there was a distinct rise in success rate as movements became completely directed. Thinking now in evolutionary terms, Hawkes speculated that insects finding themselves to one side of this 'fitness trough' (the low success rate at intermediate levels of directionality) would be selected for greater degrees of randomness in searching behaviour, while insects to the other side of the trough would be selected for greater directionality.

Though the kind of models formulated by Hawkes are preliminary in nature, they demonstrate rather well the profound influence that patterns of host plant distribution may have on the ecology and evolution of searching patterns in insects. The

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movement patterns of insects and the spatial dynamics of plants are clearly both subjects that merit further study.

Growth and survivorship on host plants

From consideration of how insects locate food plants the discussion turned next to questions concerning the suitability of host plants for growth and survival of immature stages and, in particular, the extent to which the most suitable host plants are those which are most susceptible to attack in the field. Van der Meijden summarised some of his field studies of Dutch populations of ragwort, *Senecio jacobaea*. This is an ephemeral biennial plant of unpredictable occurrence but nevertheless subject to periodic defoliation and local extinction as a result of attack by larvae of the cinnabar moth, *Tyria jacobaeae*. Among 100 populations of ragwort under study, infestation by the cinnabar moth was correlated strongly with the amount of plant biomass available in a population. A similar correlation was found between the biomass of individual plants and their susceptibility to attack. As plants or plant populations increase in biomass, so does their likelihood of being attacked. Van der Meijden drew attention to similar results obtained by others, in particular the work of Chaplin (1980) showing that the milkweed (*Asclepias*) species actually used by milkweed bugs, *Oncopeltus fasciatus*, in the field are only those whose individuals provide adequate seed biomass for growth and development.

Since availability of plant biomass accounted for only about 50% of the variance in attack on the ragwort populations, other factors must also be involved. The presence of flowering individuals was found to increase the probability of attack by the cinnabar moth. Inclusion of flowering parts in diets improved larval growth, adult emergence and fecundity, suggesting that food plant quality might be a second important factor in the yearly redistribution of the insect. No correlation was found between average nitrogen content of plant populations and insect attack. However, a more detailed analysis revealed that leaves selected for oviposition by moths contained significantly more protein than did comparable control leaves on nearest neighbours if the protein content in dry matter of the control leaves was less than 180 g/kg. On the other hand, the leaves chosen by female moths contained significantly less protein than the control leaves if the protein content of the controls exceeded 180 g/kg. The moths seem to be selecting leaves that are characterised by a rather narrow range of protein contents.

That the moths do not invariably select foliage with the highest protein content could be a consequence of the positive correlation between protein and alkaloid content among the plant populations. A significant negative correlation between alkaloid content of plant populations in one year and the frequency of oviposition by the cinnabar moth in the following year suggests that alkaloids have an increasingly negative effect on larval growth and survivorship at higher concentrations. Van der Meijden suggested that patterns of insect attack may represent a compromise between availability of plant biomass, the beneficial effects of high protein content and the deleterious effects of high alkaloid content. The intermediate protein levels associated with leaves chosen for oviposition could represent that combination of

high enough protein and low enough alkaloid content which leads to optimum growth and survivorship under natural conditions. Though the moths appear to be able to assess plant protein content, or something correlated with it, van der Meijden indicated in response to a question that nothing is yet known as to possible behavioural responses to alkaloids.

When introducing his topic, van der Meijden had re-emphasised a point made by others both before and during this symposium, namely that patterns of host selection by phytophagous insects can rarely if ever be explained in terms of a single environmental parameter. The insect and its host plant are components of a more complex world and host selection usually reflects compromises not only between factors associated with host plant quality but also between these and such 'external' factors as competition with other insects and the risk of attack by predators and parasites. A beautiful illustration of quite how complex an insect-plant interaction can become is that of the community of insects dependent on flower galls of the cruciferous plant *Diplotaxis muralis* in southern Italy. Solinas described in detail how these galls, which prevent normal flower development, result from responses of the plant tissues to active compounds originating from the saliva of the gall midge *Paragephyraulus diplotaxis* Solinas. The nutritive tissue produced as a result of gall formation provides food not only for the larvae of the gall midge but also for two other insects, a thrips and another species of gall midge, that colonise some of the developing galls. The primary gall maker is attacked by two species of hymenopterous wasps that develop as endoparasites within the larvae and two further species of Hymenoptera that are ectoparasites of the larvae of both gall midge species, namely the primary gall maker and the inquiline.

Evolutionary changes in host-plant relationships

Discussion now turned from strictly ecological aspects of existing insect-plant interactions to a consideration of evolutionary changes in host relationships. Southgate described some of the intriguing host shifts that have occurred among seed beetles of the family Bruchidae. Though the majority of bruchid species are associated with the family Leguminosae, there are some interesting exceptions. Of the fifty or so species in the genus *Caryedon* in Africa, for example, most feed on *Acacia* species in the Leguminosae but there is a group of five species that attack only the seeds of *Combretum* species in the family Combretaceae. Since the only obvious characteristic shared by the Leguminosae and Combretaceae seems to be enclosure of the seeds in similarly shaped pods, the host shift or shifts to *Combretum* could have been catalysed by the use of pod shape or texture as host recognition cues by ovipositing females. Confusion of chemical cues, another possibility, is hard to evaluate since appropriate chemical comparisons between *Combretum* and *Acacia* have apparently not been made.

Host shifts have occurred more frequently within the Leguminosae, especially as a result of human activities. For example, pigeon pea, *Cajanus cajan*, originated in southern Asia but has been introduced to many other parts of the tropics. In the Caribbean region it has now been colonised by three bruchid species, in east Africa by three other species, and in India by four species. In the first two areas, at least,

the beetles could have no experience with the plant prior to its introduction by man (Southgate, 1982). Another example is that of the groundnut seed beetle, *Caryedon serratus* (Ol.), which was introduced from Asia to Africa along with its original host, *Tamarindus indica* (tamarind). Since reaching Africa, the beetle has colonised several indigenous trees (*Acacia, Cassia,* etc.) and, in western Africa, the groundnut, *Arachis hypogea*, on which populations have reached epidemic proportions. The seed chemistry of *A. hypogea*, interestingly enough, is entirely different from that of the tree species attacked by this beetle. Southgate emphasised that he was presenting these rather dramatic examples of host shifts to stimulate thought as to the physiological and ecological conditions that might have brought them about.

Zwölfer next discussed the contrasting evolutionary trends leading to generalist and specialist strategies in phytophagous insects. Generalists are polyphagous, mobile, and opportunistic; they tend to spread their risks and are relatively unpredictable as resources for specialised predators and parasites. They lie towards the r-selected end of the r-K continuum and lack the well-defined niche differentiation associated with interspecific competition. They tend to be components of food webs that are highly variable in structure. Specialists, by contrast, are stenophagous and less mobile so that the host plant constitutes their microhabitat; the stability of interaction with the host plant leads to more subtle adaptations to the environment associated with the plant. They are more predictable to specialised enemies and are often associated with a complex of parasitoids as part of relatively stable food webs. Niche differentiation is comparatively advanced, perhaps due to interspecific competition, and specialists tend to be K-strategists.

Zwölfer went on to discuss the different impact of generalist and specialist insects on their food plants in terms of 'Parkinson's Principle', namely that increasing complexity of interaction leads to decreasing productivity of efficiency in exploiting a resource. Whereas generalists, associated with relatively unstructured food webs, can have a major impact on their food plants, specialist insects, within their naturally coevolved communities, are relatively benign. He demonstrated the contrast between complex and simple food webs by comparing some of his own work on the insects of thistles in their original European habitat with the history of introductions of the same insects into North America. Damage levels are substantially greater in the simpler introduced systems, especially where the thistles are in competition with grasses, than occur typically in the more complex food webs present in the areas of origin.

Kennedy asked Zwölfer what might be the difference between the r-K axis and the generalist-specialist axis; they seemed remarkably similar. Zwölfer agreed that application of the two axes to phytophagous insects produces basically similar patterns though there are some differences in detail. The r-K contrast applies *primarily* to reproductive effort; r-strategists are under especially strong selection for high reproductive effort and may periodically overshoot the carrying capacity of the local environment, whereas K-strategists are under relatively stronger selection for efficient exploitation of resources and exceed their carrying capacity only rarely. The generalist-specialist axis includes not only the above ecological attributes but also others such as mobility.

Replying to a question by Norris, Zwölfer indicated that associations with sym-

biotic organisms would be expected to be more frequent among specialist than generalist insects because of the many elaborate adaptations required for their maintenance and transmittal from one generation to the next. In answer to a question by Finch, Zwölfer suggested that agricultural systems should favour generalist rather than specialist insects because of the unpredictability associated with agricultural habitats.

Rowell was unfortunately called away from the symposium before this discussion but described in a written contribution some of his recent studies of host preferences among Central American grasshopper species of the genus *Rhachicreagra* Rehn (Acrididae). At least 15 of these wingless species inhabit light-gaps in the tropical wet forest of Costa Rica. Field observations and microscopic analysis of frass showed that each species attacks only one or a few forb species under natural conditions, the host plants occurring variously in the Compositae, Amaranthaceae, Umbelliferae, Urticaceae and Phytolaccaceae. Spatially overlapping species tend to attack unrelated plants. Surprisingly, feeding trials with captive individuals showed that all species tested would accept the full range of host plants attacked by the genus as a whole, but would reject plants outside this range. Though the individual species behave as narrow specialists, therefore, each retains more generalised potential. Such flexibility may explain the evolutionary 'success' of the genus since *Rhachicreagra* species should have a greater chance of colonising neighbouring but ecologically different habitats than would less flexible species. That the genus consists of a mosaic of local specialist species rather than a single widely distributed species of more generalised feeding habits can probably be ascribed to the transient, patchy nature of the habitat. This leads to small, isolated populations of low mobility and limited potential for gene flow between them.

General discussion

The open discussion centred chiefly around the use and abuse of terminology and scientific method. Wasserman expressed concern that terms like 'apparency' and 'induction' seem to be used by different people in different ways. The term 'apparent', in particular, is frequently used to describe plants that are conspicuous to a human observer but may not be at all apparent to an insect that does not search visually. I agreed that the term has sometimes been used inappropriately by ecologists, including myself, and that one cannot assume without supporting evidence that a given plant is more or less apparent than another to any given species of herbivore or pathogen. I chose the term 'apparency' to describe the susceptibility of plants to discovery by enemies (Feeny, 1976) because the word already exists in the language, it is less cumbersome and more accurate than terms such as 'discoverability', and because it does not have as strong a visual connotation as do terms like 'conspicuousness'.

Wasserman further questioned the usefulness of the concept of apparency in ecology by suggesting that the enemies of supposedly unapparent plants become so well adapted to finding their hosts that the plants become just as apparent as other plants. In other words, all plants are more or less equally apparent to their enemies.

In reply, I used insect examples to demonstrate more clearly my opinion that organisms of a given taxon can differ markedly in their apparency to enemies. Some insects are cryptic and, because they tend to be palatable, rely for survival on being relatively unapparent. Other insects are aposematic and, because they tend to be distasteful, actually benefit from enhanced apparency. The evolution of melanic forms of the peppered moth, *Biston betularia*, in industrial areas would probably not have occurred had the light-coloured and dark-coloured forms been equally apparent to predators. Finch added that he has used the term apparency to refer to the different susceptibilities of individual crop plants to discovery by insects; at any one time the plants in a field differ in apparency as a consequence of differences in growth.

Van der Meijden expressed similar concerns to those of Wasserman. Too many generalisations and sweeping hypotheses are being put forward that are not testable. As an example, he cited the concept of r and K selection, which was not set out in a testable form. Zwölfer did not agree with this and mentioned that he had been testing some of the hypotheses relating to r and K selection. As for apparency, he felt that it was perfectly possible to test hypotheses such as that thistle plants on the edge of a patch are more apparent to weevils than are those in the middle.

This discussion seemed to reflect a division of opinion that had surfaced several times during the symposium. Some of the participants felt strongly that the tendency to generalise about insect-plant interactions has gone too far. Others felt equally strongly that generalisations are necessary to stimulate and guide research in promising directions. Clearly, we investigators are spread out along our own specialist-generalist continuum. Though extremes at either end should probably be avoided, it seems to me that such diversity in conceptual approach is healthy and probably vital for the most rapid development of our subject.

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Evolution

Patterns and driving forces in the evolution of plant-insect systems

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Abstract

This contribution gives a summary of functions between higher plants, phytophagous insects and their entomophagous enemies. Four basic patterns in plant-insect systems are discussed. Evolutionary conservatism i.e., a tendency to maintain certain associations through evolutionary time, can be found if the host spectra of insect taxa of different phylogenetic age are compared. Evolutionary opportunism explains the increase of species richness with plant biomass or distribution area. Plant taxa often constitute platforms of adaptive radiation in insects. Plant components or structures offer an alternative possibility of radiation processes. The relationship between average species packing and taxonomic isolation is shown for the association of Cardueae flower heads and insects. Data on the pathways by which niche differentiation took place in Cardueae insects are given. The final section discusses possible feedbacks between plants and insects.

Key-words: evolution, generalists, specialists, speciation, food preferences, Cardueae

This contribution deals with plant-insect systems formed by angiosperm hosts, phytophagous insects and their entomophagous enemies. One of the striking features of these plant and insect groups are their extraordinary speciation rates, which have produced families including up to 40 000 and more species, e.g., Asteraceae, Chrysomelidae, Curculionidae, Ichneumonidae. Another, often ignored feature is the tremendous food-web complexity in plant-insect systems. A third feature is the diversity of chemical and morphological structures, of physiological adaptations, behaviour and life history patterns found in these communities. An analysis of the structure of plant-insect systems and of the driving forces in their evolution is hence of a broad interest for ecological and evolutionary theory.

Following a few early approaches, e.g., Southwood's (1960) investigation of species/area relationships of insects associated with Hawaiian trees, or the study by Ehrlich & Raven (1964) of the coevolution of butterflies and plants, there is now a fast growing body of literature dealing with the structure and evolution of plant-insect systems. Price (1977), Strong (1979), Zwölfer (1978) and others have reviewed this literature. The impression emerges that plant-insect systems have some regularity in their structure. We are, however, far from recognizing predictable patterns

other than the well documented species/area phenomenon, which according to Strong & Levin (1979) statistically accounts for 20% to 60% of the variation of phytophagous species richness. Obviously, we need much more information on the structure of herbivore guilds, their interactions and their degree of species packing, we need more information on food webs, niche differentiation and grazing pressure on the host and we need many more data on the phylogenetical history of plant and insect taxa. My contribution to this symposium, biased as it may be by my own fields of research, intends to emphasize some of the problems.

Functions in plant-insect systems

The biological and ecological relationships between plants, phytophagous insects and entomophagous insects form the basis for the understanding of plant-insect systems. As I have discussed this in more detail elsewhere (Zwölfer, 1978) only a short summary of these relationships will be given here.
— For many phytophagous and some entomophagous insect taxa plants constitute whole microhabitats rather than mere food resources. In addition to their trophic function they provide recognition tokens, shelter, and rendez-vous places for male and female insects. Thus, they can contribute to the canalization of gene flow and to reproductive isolation (Bush, 1975).
— Plant structures can be arenas for competitive interactions of the insect species exploiting them, just as the body of a phytophagous host insect can be the arena for competing parasitoid larvae. On both levels, a competitive inferiority in the case of direct contact of phytophagous or entomophagous larvae can be counterbalanced by a superiority of adults in host or host plant finding and/or distribution of eggs. This phenomenon of a mutual trade-off between larval and imaginal superiorities is an important basis for the coexistence of species in guilds of phytophagous and entomophagous insects (Zwölfer, 1980).
— Token stimuli of the host plant often guide the orientation scheme of entomophagous insects.
— Host plants are the bases of often complex food webs, which in turn can reduce the pressure of their phytophagous exploiters.
— As has been experimentally shown by a number of successful projects of biological weed control, phytophagous insects can have a latent potential to lower the competitive capacities of their host plants.
— Phytophagous insects exert a selective pressure that promotes the diversification of defence mechanisms in plants. On the other hand, this process stimulates the evolution of new types of physiological adaptations of insects to plants and of new forms of exploitation of plants by insects. A similar evolutionary interaction can be found between phytophagous hosts and some of their entomophagous parasitoids.

Paleobiological data

Smart & Hughes (1972), Kevan et al. (1975) and Zwölfer (1978) have reviewed the paleontological data relevant to the evolution of plant-insect systems. Saprophagous insects, insects feeding on spores or pollen and sucking insects date

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back to the late Paleozoic period. Sucking plant tissues, which according to Smart & Hughes (1972) possibly was already developed by the Paleozoic insect order Paleodictyophora and stimulated by the anatomy of the Cordaitales, is obviously the first really phytophagous way of life. In the Permian period ancestors of plant sucking hemipterous taxa are well documented (Hennig, 1969) and feeding holes on fronds of ferns indicate the existence of chewing phytophagous insects. The Tenthredinidae, which appear in the Triassic period, are among the first phytophagous insect taxa adopting this type of feeding. Until the late Jurassic period the evolution of phytophagous insect taxa, e.g., the Coleoptera families Curculionidae and Chrysomelidae, made little progress. In the late Mesozoic period, however, triggered by the explosive evolution of angiosperm taxa, numerous phytophagous and entomophagous insect taxa and new trophic strategies appear, e.g., the formation of plant galls, the exploitation of host insects. This trend continues during the Tertiary epoch, which gives rise to such modern phytophagous taxa as bark beetles (Scolytidae), gall wasps (Cynipidae), phytophagous gall midges (Itonididae), leafmining flies (Agromyzidae) or fruit flies (Tephritidae). Obviously the majority of the present plant-insect systems have evolved during the late Cretaceous and Tertiary epochs, which have been periods of tremendous adaptive radiations in phytophagous and entomophagous insects.

The main evolutionary patterns of plant-insect systems

If the available data on plant-insect associations are analyzed, at least four basic patterns emerge that per se or in various combinations, contribute to explain the present picture.

Evolutionary conservatism Complex successions of radiation processes have obscured much of the original plant-insect associations, but nevertheless we can still find some relict relationships. Figure 1 analyzes the data on the host spectra of 2488 European phytophagous insect species (Adelgidae and Phylloxeridae: 32 spp.; Coccidoidea: 23 spp.; Tenthredinidae: 277 spp.; Apionidae: 124 spp.; Curculionidae without Apionidae: 393 spp.; Chrysomelidae: 222 spp.; Geometridae: 152 spp.; Tortricidae: 210 spp.; Gelechiidae: 150 spp.; Noctuidae: 40 spp.; Pyralidae: 84 spp.; Lachnidae and Aphididae: 478 spp.; Agromyzidae: 184 spp.; Tephritidae: 119 spp.) to test the hypothesis that the phylogenetic age of a phytophagous taxon influences its host plant spectrum.

Three of the 14 insect families are phylogenetically ancient groups (Adelgidae/ Phylloxeridae, Coccidoidea, Tenthredinidae) as they belong to taxa already recorded from the early Mesozoic era. Three other families (Apionidae, Curculionidae, Chrysomelidae) belong to taxa first recorded from the late Mesozoic era. The origin of the remaining 8 families (Geometridae, Tortricidae, Gelechiidae, Noctuidae, Pyralidae, Lachnidae and Aphididae, Agromyzidae, Tephritidae) is in the Cretaceous and Tertiary period i.e., they are phylogenetically young. The graph shows the proportion of phytophagous species associated with phylogenetically ancient vascular plant groups (Pteridophyta and Equisetales; Coniferae; Angiospermae: Magnoliidae; Angiospermae: Rosidae and Hamamelidae) and with phy-
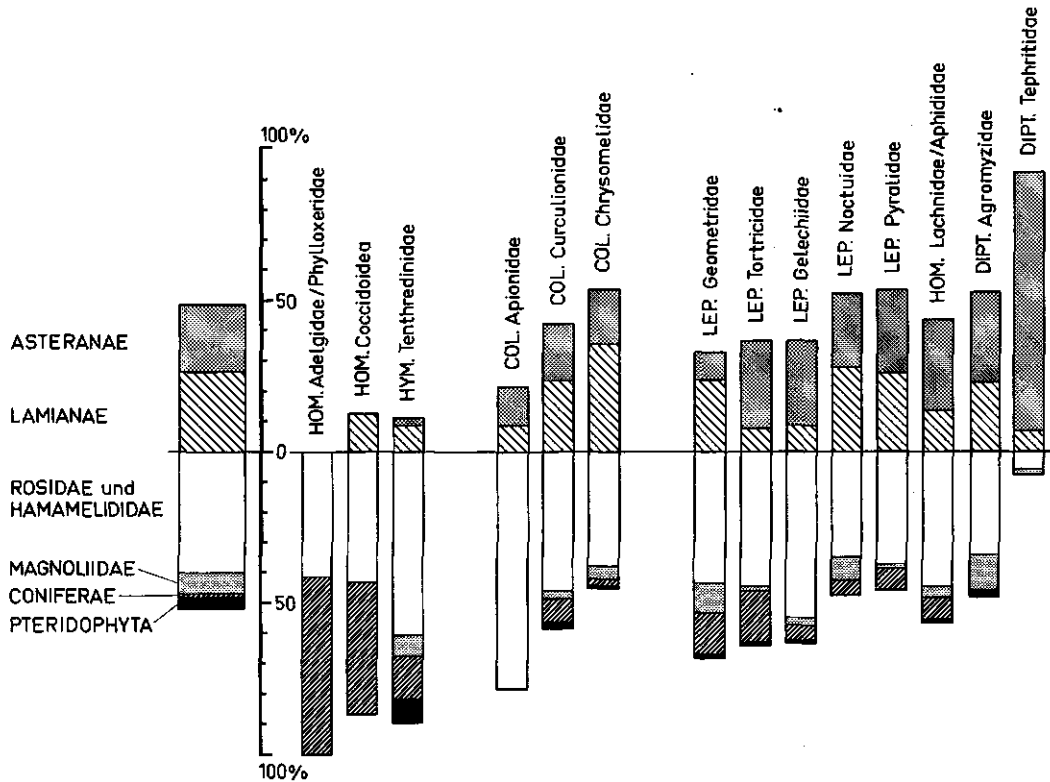
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Fig. 1. Proportion of phylogenetically ancient and young plant taxa in the host spectra of 14 families of phytophagous insects (for explanation see text).

logenetically young groups (Angiospermae: Lamianae; Angiospermae: Asteranae). The proportion in which these plants (together 1490 species) are represented in the middle-European flora is indicated at the left side of the graph. The plant data are taken from Takhtajan (1973) and Ehrendorfer (1971), the host plant data have been compiled from the standard literature dealing with European insect orders.

The difference between the host spectra of the early Mesozoic phytophagous group and the phylogenetically younger insect groups is statistically highly significant: ferns and/or conifers are attacked at a much higher proportion and the most modern angiosperm group, the Asteranae, in a much lower proportion than is the case in phylogenetically younger taxa. The Tephritidae, a very young family, the origin of which dates back to the middle Tertiary period, has a statistically significant higher proportion of Asteranae (the most modern angiosperm group under consideration) in its host range than the remaining 13 phytophagous families. These data suggest that phytophagous insect groups, even through evolutionary time, tend to maintain some parts of their ancient associations and that modern phytophagous groups on the average adopted modern plant groups as host more readily than older

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groups. A similar conclusion can be drawn if old taxa of the Coleoptera (Cupepidae, Belidae, Oxycorinae, Nemonychidae), the Hymenoptera (Siricidae, Orussidae, Xyelidae), the Lepidoptera (Micropterygidae, Cossidae) and the Diptera (Pachyneuridae) are evaluated with regard to their food preferences.

Evolutionary and ecological opportunism It has been demonstrated by many authors that there exists a relationship between the abundance of a plant species and the richness of its phytophagous insect species. Southwood (1961) showed this for trees. Kovalev (1968) claims that the principle of 'host dominance' is responsible for the distribution of gall formers in the northern hemisphere: plants such as oaks and willows, which dominated the vegetation during the last geological periods, show the highest number of gall-forming insect species. Lawton & Schröder (1977) found a clear relationship between species richness of phytophagous insects (measured as ln of the species number S) and the size of the distribution area (measured as ln area) in perennial herbs, woody shrubs, monocots (excluding grasses), and weeds and other annuals. In an analysis of the species richness of agromyzid flies on British Umbelliferae, Lawton & Price (1979) found that the correlation with the geographical range of the individual plant species is highly significant. Additional examples are discussed by Strong (1979). For a given geographical range, species richness of phytophagous insects significantly increases with the growth type of the host: trees tend to have more insects than shrubs, and shrubs have more insects than herbs (Lawton & Schröder, 1977; Strong & Levin, 1979; Strong, 1979).

For this relationship three explanations are offered: a larger distribution area (or a larger plant species) provides more resources and may support larger insect populations, there exists a higher degree of habitat heterogeneity or structural heterogeneity; or chances for 'passive assembling' are increased (Strong, 1979). These three possibilities which are not mutually exclusive, can be regarded as 'evolutionary opportunism', if an increasing rate of chance contacts with a new plant species leads to the adoption of and concomitant adaptations to new host species. It may be 'ecological opportunism' if insects simply exploit hosts constituting a larger resource without particular changes in their host selection schemes.

However, one has to agree with Strong (1979) who states that the species/area relationship is a rather trivial phenomenon in community geography and that 'species richnesses need special explanation only when they deviate from the species/area relationship appropriate for the region and the organism in question'. I found such deviations when reexamining the insect complexes of European Cardueae already analysed by Lawton & Schröder in 1977. This is not a literature-based investigation but the evaluation of plant-insect data that I have systematically collected during the last 20 years. At present, data on 944 flower head populations of a total of 65 European Cardueae species are available for regression analyses. I have examined the influence of various plant parameters on average species packing, total species richness, species richness of euryphagous insects, and species richness of stenophagous insects (Table 1).

Average species packing is the average number of phytophagous species (gall midges excluded) found when dissecting a 'population' of thistle flower heads i.e., usually a group of 100 heads collected from a particular Cardueae species at a given

Table 1. Correlation coefficients of species packing (average number of phytophagous species in the flower head population) and species richness (total number of phytophagous species found in the heads of a host plant species) and various host parameters. Data from 56 Cardueae spp.

Independent variables	Species packing	Species richness (stenophagous spp.)	Species richness (euryphagous spp.)	Species richness (all species)
Log number of host populations investigated	0.14 (0.02)	0.59** (0.35)	0.75** (0.56)	0.86** (0.74)
Number of regions	0.06 (0.00)	0.42* (0.18)	0.73** (0.53)	0.74** (0.55)
Number of spp. in host plant genera	0.52** (0.27)	0.12 (0.01)	0.18 (0.03)	0.20 (0.04)
Size of flower head (mm)	−0.23 (0.05)	0.08 (0.01)	−0.01 (0.00)	0.02 (0.00)
Life type of host plant	0.07 (0.00)	0.12 (0.01)	0.10 (0.01)	0.04 (0.00)
Type of habitat	0.03 (0.00)	0.05 (0.00)	−0.07 (0.00)	0.09 (0.01)
Number of spp. in host subtribe	−0.07 (0.00)	0.02 (0.00)	0.27 (0.07)	0.24 (0.06)

** Correlation significant at $P = 0.001$.
* Correlation significant at $P = 0.01$.
Data in parenthesis: coefficient of determination (r^2)

station. In contrast to species richness, which is a compiled list of insects over the whole area of the plant, species packing gives an exact picture of the size of a phytophagous guild exploiting simultaneously a real plant population. Lawton (1976) has provided similar data for insect guilds on bracken and Ward (1977) did the same for juniper in southern England.

Stenophagous insects are species associated with only one or a small group of closely related congeneric Cardueae species. Euryphagous insects are such species that I have bred from Cardueae genera or subtribes. The great majority of these euryphagous species, however, restrict their host spectrum to the plant tribe Cardueae or the family Astereae. Hence, they are still fairly specialized when compared to so-called polyphagous insects which may exploit several plant orders.

As is shown in Table 1 evolutionary opportunism is not a very satisfactory explanation for the patterns found in the phytophagous guilds associated with the flower heads of European Cardueae. The numbers of populations investigated (i.e., the sampling effort) and the numbers of geographical regions (as defined by Zwölfer, 1965) assessed by the sampling programme are not correlated with species packing (i.e., the average guild size) and they are significantly less correlated with species richness of specialized insects (stenophagous species) than with species richness of generalists (euryphagous species) or that of all phytophagous species. This suggests

that mainly non-specialized phytophagous insects are involved in the species/area relationship. The fact that the size of flower heads i.e., the dimension food resource, is not correlated with species richness is also an indication that evolutionary opportunism was not a major driving force in the history of these plant-insect systems. Otherwise the capitules of Cardueae species with large flower heads (e.g. artichoke or *Silybim marianum*) should be inhabited by more phytophagous species than Cardueae with small heads (e.g. diffuse knapweed or creeping thistle). Actually the insect species attacking Cardueae heads (e.g., weevil species, tephritid species, or Microlepidoptera) have adapted their body size to the dimension of the flower heads of the host species attacked rather than concentrating on hosts with large heads.

There is no doubt that evolutionary opportunism i.e., the trend to adopt host plant populations distributed over large areas or having a high biomass, does exist in phytophagous insects, but it may be mostly shown by generalists. It seems to be insufficient to explain the composition and size of phytophagous guilds, in the sense of constellations of coexistent and coadapted species, as they occur in Cardueae heads (Zwölfer, 1975).

Plant taxa as radiation platforms Adaptive radiation of phytophagous insects may occur on the same plant species, where different organs are then exploited by closely related insect species. Such a type of speciation with a concomitant transfer to new plant structures can be found in any phytophagous insect order but it is particularly frequent in cynipids. Much more common, however, are radiation processes that took place on the platform of taxonomically related plants. As related plant species usually offer similarities with regard to structural and chemical components, the adoption of such species will require less transformations of the biological adaptations and chemosensory mechanisms than when an entirely unrelated plant is colonized by the insect. Therefore, transfer to taxonomically related plants and the resulting pattern of species groups, genera or even tribes of phytophagous insects associated with and confined to closely related host plants, are a characteristic feature of plant-insect systems.

In Figure 2 this is shown for phytophagous insects living on European Cardueae. By examining the taxonomic relationships and the biologies and host ranges of congeneric phytophagous insects, it was possible to state for 77 species whether the actual host relationships were due to a simultaneous change of host species and exploited structure (4% of the assessed cases), to a change of the structure only (8%), or to a change of the host species only (83%). In four species (5%) a change of the host plant occurs only at localities where the original host is occupied by an ecologically homologous competitor (ecological character displacement).

If plant taxa serve as platforms for adaptive radiation of phytophagous insects, increasing size of a plant taxon should increase speciation rates of specialized phytophagous insects. Moreover, the 'exchange' of insect species should be facilitated in large plant taxa. On the other hand, taxonomic isolation (number of congeneric relatives) should result in an impoverished insect fauna. Lawton & Schröder (1977), Lawton & Price (1979) and Neuvonen & Niemelä (1981) have investigated the question whether there is a correlation between species richness of phytophagous insects
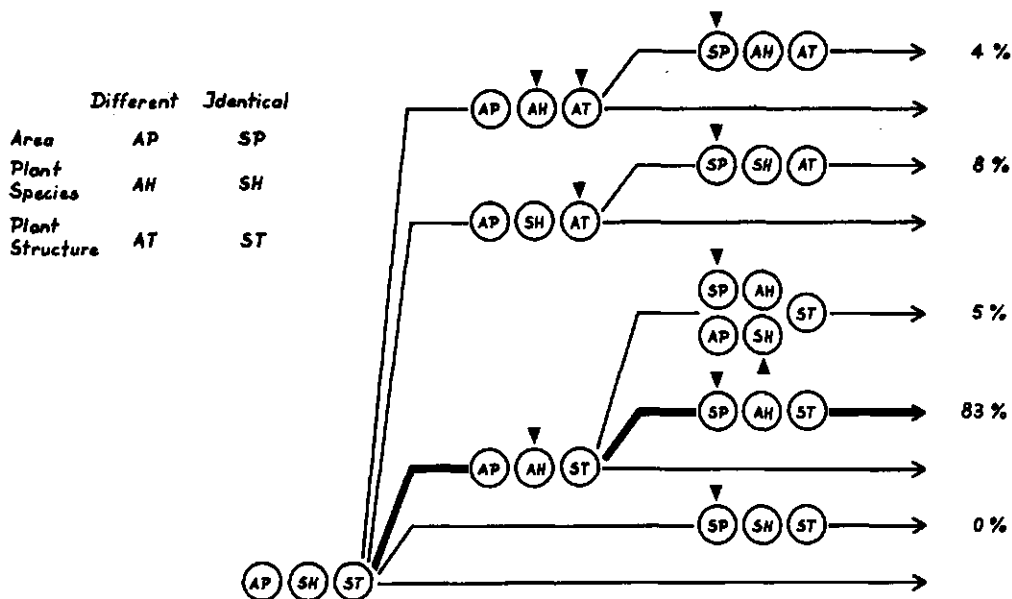
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Fig. 2. Speciation and niche differentiation in 77 insect species associated with Cardueae hosts. Percentages refer to the proportions of species which followed a particular pathway of differentiation. Black triangles indicate changes in sympatry/allopatry, host plant species or exploited plant structure.

and taxonomic isolation of the host. So far only a few cases (British monocots, Typhlocybinae leafhoppers on British trees) and only slightly significant correlations have been demonstrated. In the majority of data sets (Umbelliferae, perennial herbs, woody shrubs, weeds and other annuals, total insect fauna of Cardueae, Finnish deciduous trees and shrubs) statistically significant correlations between species richness and taxonomic isolation could not be found. It is remarkable that in the insect complexes associated with Cardueae heads species packing (but not total species richness of specialists or generalists) provides a statistically highly significant correlation with taxonomic isolation (Table 1). I assume that species packing i.e., the average size of true guilds, is a better measure of the degree of adaptive radiation and exchange of phytophagous insects than species richness, which is based only on compiled lists of insects and which certainly includes many species associated only very marginally with the given plant species. Hence, attempts should be made by community ecologists to obtain more data sets on species packing.

Plant components or structures as radiation platforms Some insect groups obviously have developed trophic strategies to exploit particular plant structures or orientation mechanisms attuned to particular token stimuli. The host spectrum of such insect taxa may contain taxonomically unrelated plants which, however, have a key component in common. Fruitflies of the genera *Rhagoletis* or *Dacus* attack a large array of angiosperms. Many hosts are taxonomically unrelated but have simi-

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lar types of fruits, which constitute the key component for genera such as *Rhagoletis* or *Dacus*. The weevil genus *Smicronyx* radiated on angiosperm genera that are unrelated, but all are parasitic plants. The adaptive radiation of the weevil genera *Bagoous* and *Phytobius* proceeded on unrelated aquatic plants. These are only a few examples, and many more can be provided if the plant chemistry is taken into consideration.

Feedback in plant-insect systems

Biological control operations with phytophagous insects (weed control) or entomophagous insects (insect pest control) have demonstrated that in both groups of insects there are species that can control their host population. This occurs either by a more or less density-dependent numerical reduction of the host, or by a reduction of the competitive capacity of the host plant. In food webs the latent control potential of phytophagous and entomophagous insects is usually compensated, particularly if there is a heterogeneous environment and if the host plant does not form monotonous stands. An increase in plant density will bring their populations under an increased grazing pressure, as is shown by the susceptibility of monocultures to insect pests. Similarly a continued increase of a phytophagous population will result in a growing pressure of entomophagous species. These latent control mechanisms help to maintain species diversity on the level of the plant and of the phytophagous insect. However, this system of mutual checks in plant-insect communities will never provide complete stability, as unpredictable changes in the abiotic environment (e.g., short-term changes of meteorological conditions or long-term changes of climate) steadily interfere. Moreover, there is a continuous selection premium to escape ecological control mechanism by evolutionary processes. Ehrlich & Raven (1964) have been among the first to discuss the consequences of this evolutionary escape: an increasing diversification of defense mechanisms in plants promotes the evolution of new exploitation strategies in insects. Similar evolutionary interactions can be found between phytophagous host insects and entomophagous parasitoids (Zwölfer, 1978). The interplay of diversifying defence mechanisms and diversifying exploitation strategies seems to provide systems of positive feedback loops. Together with the isolating effect of host plants on their insect inhabitants it must have contributed to the explosive speciation in higher plants, phytophagous and entomophagous insects.

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Host shifts, genetic models of sympatric speciation and the origin of parasitic insect species

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Abstract

Host shifts in parasitic insects may involve changes in host selection and survival genes. These shifts occur most often when the old and new host grow sympatrically and usually require genetic changes in both host selection and survival genes. Several proposed genetic modes of sympatric speciation are assessed with respect to their relevance to host race formation and speciation in parasites. By assuming random host selection and ignoring the fact that parasites frequently mate on their hosts these models are inappropriate for host-parasite systems. An alternative model of sympatric host race formation and speciation is proposed which incorporates more realistic assumptions about host and mate choice which significantly alter the pattern of gene flow and selection. Other ecological and genetic factors that influence host shifts are also considered. From this perspective host races and species are likely to arise sympatrically in a wide range of parasitic organisms.

Key-words: host race formation, speciation, genetic models, evolution

It is now a well recognized phenomenon: a plant introduced into a new area frequently remains free of serious pests and other organisms for varying lengths of time. Inevitably, however, the new plant succumbs first to a trickle, then to a flood of invading species. Eventually a more or less stable biotic guild develops consisting of insects, nematodes, fungi, and microorganisms all interacting at various levels of complexity and importance. At this point the new host may have acquired an array of new host races and even species that have evolved during the course of adaptation. In some aspects the process resembles succession in plant communities. In this paper we wish to focus from a genetic perspective on the conditions under which such host shifts occur and how they may lead to host race formation and speciation.

We define a host race as a population of a species living on and showing a preference for a host which is different from the host or hosts of other populations of the same species. Host races represent a continuum between forms which partially interbreed to those that rarely exchange genes (modified from Bush, 1969). We view parasites in the general conceptualization of Price (1980) to include a wide variety of species which gain their resources from other species, usually to the latter's detriment. The term parasite would encompass a range of life forms from microorgan-

isms and fungi to arthropod parisitoids of plants and animals. Parasitic species thus defined make up a significant percentage of the world's faunal diversity (Bush, 1975).

There are four adaptive genetic scenarios that might occur once a new host plant is introduced into an area. The following genetic changes may be required for host shifts:
— none
— survival gene only
— host recognition gene only
— host recognition and survival genes

We will discuss the evolutionary consequences of each case separately and then focus on alternative genetic models of sympatric host race formation and speciation.

Adaptive genetic scenarios

Case I. No adaptive genetic changes required for host shift If the parasite is capable of recognizing and surviving on the new host plant, then the host range of the insect will simply be expanded to include the new host. No new mutations need to occur at least initially that improve survival or host selection prior to the host shift. This may be true even if the new host is of lower nutritional quality than the original host, due to an escape from competition, predation, or parasitism. Although several factors such as induction or host plant phenology might partially reduce gene flow between populations on the different hosts for non-genetic reasons, in the absence of mutation and selection which improve fitness on the new host, it is unlikely that these populations will diverge to any great degree. However, if mutations which improve host recognition and survival on the new plant do arise, host race formation and speciation could evolve. The situation then resembles Case IV discussed later.

Case II. A change in survival genes is required for a host shift In this situation, few insects or their progeny can survive on the introduced host unless a mutation occurs in a survival gene that makes it possible to develop on the new host. This type of genetic host-parasite interaction involving resistance and survival genes is well known (Day, 1974) and is best exemplified in insects by the highly coevolved gene-for-gene relationships between resistance genes in wheat cultivars and survival genes in the Hessian fly. As long as these flies mate at random and are unable to distinguish resistant from susceptible genotypes of the host plants, survival genes will be retained as a polymorphism. Host races are unlikely to evolve in the face of such gene flow unless assortative mating by host preference develops subsequent to the shift.

Case III. A change in a host recognition gene is necessary for shift to a new host plant Parasites can sometimes be forced to oviposit and feed upon a non-host species with no obvious effect on survival or fitness (Dethier, 1954; Fraenkel, 1969) suggesting survival genes are not always involved in host utilization. Yet the potential host is never used in nature even when growing side-by-side with a normal host.

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The new host in this case is rejected because it does not provide some essential host recognition cue. Alternatively, a potential host may be unacceptable because of intense competition, predation, or parasitism (Smiley, 1978; Gilbert, 1979), although not all examples can be explained in terms of such 'ecological monophagy.'

When alteration in a host recognition gene results in a shift to a new host plant and there are no differences in parasite fitness on either host, it is unlikely that sympatric speciation will occur. When closely related, host-specific species can be forced to utilize each other's host with no apparent reduction in fitness, it may be reasonable to conclude that speciation was the result of allopatric isolation or perhaps occurred via the 'runaway process' of sexual selection (Kirkpatrick, 1982; Lande, 1981).

Case IV. A change in host recognition and survival genes is required for a host shift   A shift from one host plant to another may require genetic alterations of loci responsible for both host recognition and survival (Huettel & Bush, 1972). The genetics of such a shift have been explored by Bush (1975) who concluded that host shifts of this nature are the most likely candidates for producing new host races and sibling species in parasitic groups. For this reason we will discuss the implications of host race formation involving both host recognition and survival genes in the context of previously proposed mathematical models of speciation.

Genetic models of host race formation and speciation

Several authors have developed mathematical models of the genetics of sympatric speciation (Maynard Smith, 1966; Bazykin, 1969; Caisse & Antonovics, 1978; Pimm, 1979; Felsenstein, 1981). Although none of these models were directed specifically to resolve the problems of speciation in parasites, they do relate to the problem in varying degrees. One of the most widely cited of such models is that of Maynard Smith (1966). One variation of his model involves genetically controlled host selection, with mating occurring on the host. Although this approach may apply realistically to parasites, a full analytical and quantitative treatment of the specific conditions necessary for the evolution of reproductive isolation by this mechanism was not presented. Thus, it is difficult to assess how common such a mode of speciation might be under natural conditions.

More recently, Felsenstein (1981) has developed a similar haploid model of sympatric speciation based on the interaction of two diallelic viability (survival) loci (B and C) which preferentially adapt individuals to either of two niches (host 1 and 2), and a third assortative mating locus (A) which under the right conditions may bring about reproductive isolation. By computer simulation, this model attemps to define the minimum parameters of selection intensity and genetically determined assortative mating behaviour which are necessary for the evolution of reproductively isolated populations. Its conclusion is that in the absence of linkage between assortative mating and viability loci, conditions favouring sympatric speciation may be restrictive, requiring either high selection intensities or strong expression of assortative mating genes. However, there are a number of reasons for questioning the relevance of this mathematical model to the actual biological processes involved in host

shifts by parasitic organisms.

The central problem is that this model fails to take into consideration the possible association between mate selection and host selection behaviours. It is well known that a wide range of parasitic organisms frequently utilize their host as a 'rendez-vous' site for mating and courtship (Bush, 1975; Price, 1980). In Felsenstein's model, these two processes are totally uncoupled. Individuals mate assortatively but then randomly disperse with respect to the *A* locus to either of the two hosts available irrespective of alleles at their viability loci (*B, C*). Thus, even when conditions result in the development of two distinct species, each continues to randomly select and utilize both of the available hosts equally. This occurs despite the fact that selection during each generation eliminates non-viable genotypes that happen to settle on the wrong host. Such a situation clearly bears little resemblance to proposed models of sympatric speciation via host race formation, where mate and host selection are coupled and emerging species are largely confined to the utilization of a single host (Bush, 1975).

Consider the case where genetic variability exists at a locus governing both habitat and mate preference as in many parasites. Selection can act on this locus in at least two fundamentally different ways. First, selection for assortative mating can prevent recombination between two interacting viability loci as in Felsenstein's model. However, if mate and host selection are biologically coupled activities as they are in many parasitic organisms, and host selection genes simultaneously specify mate choice, selection can operate through a second and potentially more efficacious process. An association (i.e. linkage disequilibrium) between an allele at a viability locus which confers superior fitness on one of the two hosts with an allele at a second locus specifying a preference for this same host will be directly favoured. This occurs not only due to the reduction of less viable recombinants (as in Felsenstein's model), but also because of the obvious benefit which accrues to individuals who select the host which is most suited to the alleles present at their viability loci.

Taken from this perspective, perhaps a more appropriate mathematical model of the biology of parasite host shifts is the migration modification model of Balkau & Feldman (1973). This model envisions two populations and two loci. One locus represents a viability locus, with alternative alleles each adapted to a different habitat. The second locus segregates for alleles influencing the tendency for individuals to migrate from one habitat to another. In the context of host shifts, this would be analogous to an induction gene which increases the tendency to remain on whichever host they previously fed upon. It was found that if an allele which reduced the tendency to migrate was introduced at a low frequency, it would always increase and thus bring about at least partial reproductive isolation between the two populations.

Felsenstein's model poses more severe restrictions on the development of reproductive isolation. He suggests that this is so because reproductive isolation is due to the substitution of the same allele (which reduces migration) into both populations in the Balkau and Feldman model versus the greater difficulty of substituting two different alleles for assortative mating into each of the two populations in Felsenstein's model. Although this may be true, the greater part of the difference between the two models may lie in the fact that Balkau's and Feldman's model has the

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additional advantage of coupling the ecologically important variable of mate selection with habitat preference so that genotypes remain in the environments to which they are best adapted.

Although the model developed by Balkau & Feldman (1973) represents a step in the right direction, additional modifications are necessary in order to more realistically simulate the biological properties of parasitic organisms. We wish to point out how Felsenstein's model might be modified to accommodate cases where host race formation comes about as a consequence of different host choice alleles (A,a). For the remainder of the paper we will refer to this modification as the parasite model. Since it is assumed that individuals mate on their host, such differences in host choice automatically bring about at least partial reproductive isolation. It is also assumed that prior to the introduction of the new host the allele influencing preference for this host (a), as well as the alleles at the two loci which enhance survival on the new host (b, c), are maintained in the population on the original host at low frequency by mutation pressure. At this stage in the model we also assume that the genetic control of host choice by the host choice locus A,a is imperfect and that some errors occur. We expect that partially reproductively isolated host races will evolve at this stage but more conclusive results must await a more quantitative treatment now in progress. In addition, if genetic variability exists (or arises through mutation) at a fourth locus (D, d) capable of improving host choice still further, the alleles at this locus will be directly selected for non-random association (disequilibrium) with the ecologically related alleles already present at the other loci. Thus, by direct adaptive selection for non-random association between such ecologically coupled alleles $(ABCD$ and $abcd)$ host choice mechanisms may be perfected and consequently lead to speciation in these parasitic organisms.

At this point we wish to compare the attributes of the new species formed via the Felsenstein and parasite models in order to assess their biological relevance. In the parasite model, the initial population has been split to form reproductively and ecologically isolated species with each utilizing only one host. In the Felsenstein model, however, reproductive isolation occurs without any corresponding ecological isolation as individuals continue to 'compete perfectly with each other' (Felsenstein, 1981, p. 137). The closest real biological example which we can imagine fitting the attributes of this latter model would perhaps be some kind of plankton species which is incapable of influencing into which of several alternative habitats it is passively dispersed. If this species exhibited genetic variability at viability loci correlated with the different habitats and could mate assortatively by some mechanism such as seasonality, the conditions would satisfy the assumed biological properties inherent in the Felsenstein model. However, such a creature bears little resemblance to most of the parasitic organisms for which sympatric modes of speciation have been proposed (Bush, 1975; White, 1978).

We have summarized some of the major diferences between the Felsenstein and parasite models of sympatric speciation in Table 1. Complementary to our discussion above, Taylor (1976) has pointed out the ways in which host selection (or more generally habitat or niche selection) may facilitate the maintenance of a polymorphism. This conclusion has been corroborated experimentally for *Drosophila* by Jones & Probert (1980). These results emphasize the importance of host selection to

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Table 1. Major differences between the Felsenstein and parasitic sympatric speciation models. Both models of sympatric speciation are haploid and deterministic with discrete generations, assume infinite population sizes, no linkage (initially), equal niche (or habitat or host) resource sizes, and separate density-dependent regulation of population size within each niche. In habitat or host 1, host-related viability or survival alleles *BC* are favoured, and in habitat or host 2, *bc* is at an advantage, while intermediate genotypes (*Bc* and *bC*) are of intermediate fitness in each habitat and of lower fitness on average in the total population of both habitats combined.

	Felsenstein model	Parasite model
host selection	random	non-random
mating	assortative	on host
locus *A*	assortative mating gene	host selection gene
locus *D*	assortative mating modifier	host selection gene
selection on *A*	initially weak (or negative) and indirect	initially strong and direct
selection on *D*	very weak and indirect	strong and direct

populations which utilize two or more hosts, and thus further question the relevance of conclusions derived from a model such as Felsenstein's which assumes that host selection is a purely random process.

One final weakness of the Felsenstein model which we wish to contrast with the parasite model concerns his use of assortative mating genes to bring about reproductive isolation. To begin with, it is not explicitly stated at what frequency the alleles at this locus are initially set during simulation runs with this model. And it has been pointed out elsewhere that a polymorphism for assortative mating in the initial population is likely to exist only under a restricted set of conditions (Moore, 1979). If an assortative mating allele is introduced into a population at an initially low frequency, individuals possessing this allele will be at a disadvantage due to the difficulty of finding appropriate mates (Wilson, 1982). While in the parasite model the individuals initially colonizing a new host may suffer a similar disadvantage due to absence of mates, they also experience a large counterbalancing increase in fitness due to the probable absence of competitors (Pimm, 1979; Gibbons, 1979), predators, and parasites on the new host.

There is no direct selection acting to increase a rare assortative mating allele which corresponds to these important ecological interactions. Instead, selection for assortative mating in Felsenstein's model is confined to act only by the reduction of intermediate genotypes *Bc* and *bC* whose fitness is lower on average than either *BC*

and *bc* (assuming that the viability loci interact multiplicatively with each other). Thus the selection on the locus *D,d* which in Felsenstein's model modifies (enhances) the degree of assortative mating is even more indirect since it must, in turn, act via any disequilibrium already established between *A* and the *B* or *C* viability loci. This again contrasts sharply with the more direct effect brought about by the different alleles at the *D,d* host choice locus in the parasite model. Felsenstein (1981, p.132) justifies the choice of the particular assortative mating modification mechanism used in his model '... because it places no direct selection on the modifier, and in hopes that the result will prove to be insensitive to the specific modification scheme.' In contrast, we have argued that this procedure lacks applicability in that the use of an assortative mating scheme at all (as opposed to a host choice mechanism) ignores important aspects of the biology of many organisms and especially parasites.

Despite these deficiencies, Felsenstein's model of sympatric speciation represents a considerable step forward in this field due to its thorough and quantitative approach to the modeling process. And it does serve to emphasize one very important aspect of the underlying genetic mechanism which we suspect will also apply to more realistic models of host shifts in parasitic organisms. This centers on the role of linkage among the loci involved in this process. Felsenstein found that a reduction in the recombinant fraction (i.e. increased linkage) between a viability locus (*B* or *C*) and the assortative mating locus (*A*) substantially reduced the selection intensity and degree of assortative mating necessary to bring about sympatric divergence. Linkage between the viability gene and the assortative mating gene also had the effect of promoting divergence in a similar model developed by Caisse & Antonovics (1978). On the other hand, increased linkage between the two viability loci (*B, C*) themselves made the restrictions on sympatric speciation considerably more severe in the haploid version of Felsenstein's model, but only slightly more severe in diploid versions of this model. Unfortunately, he did not examine the case where all three of these loci are linked.

Linkage relationships between host selection and survival genes can therefore play an important role in the sympatric divergence of populations utilizing different hosts or habitats. The closer the linkage the more likely distinct host races and eventually species will evolve. Such 'supergenes' arise as a result of chromosome rearrangements or transpositions by movable genetic elements which appear to be pervasive among organisms (White, 1973; Campbell, 1981). For example, it has been estimated that about one individual in five hundred is heterozygous for a new chromosomal rearrangement in organisms as diverse as lilies, grasshoppers, and man (White, 1978).

Although there are many ways linkage patterns can be established, it is not necessary to postulate that a chromosomal rearrangement bringing about tight linkage between these loci must arise de novo in each successive host shift. In the course of previous host shifts and speciation events in a parasite's evolutionary history, selection would have favoured close linkage of genes governing such ecologically correlated traits as host selection and host-associated viability differences. The lack of adequate genetic variability of linkage relationships among loci would not, therefore, be expected to exert serious constraints on the sympatric development of re-

productive isolation. However, differences in patterns of linkage between host selection and viability genes may to some extent determine whether a parasite is monophagous, oligophagous, or polyphagous.

Other factors that influence host shifts

There are two ways a parasite can encounter and colonize a potential new host plant. Wind or some other agent can transport a parasite to a new area where it may, under favourable conditions, infest a new host plant. Alternatively, it can shift to a new host that has been introduced or has been growing in close proximity. Although long-distance transport and colonization of a new host have occurred, such events require special conditions. Not only must the insect be preadapted to recognize and survive on the new host, it must also be able to compete with the local fauna for resources. In parasites, many of which are monophagous (Price, 1980), it is far more likely that successful host shifts occur when a host plant is introduced into a new area free of its associated fauna and flora. Under these sympatric conditions local parasites would have opportunities to colonize and test their ability to survive on the new plant repeatedly during and after acquiring the right combination of host recognition and survival genes. Many attempts probably end in extinction and these events must be common following the introduction of a new host plant into an area (Bush, 1975).

There are several other genetic and ecological factors influencing host race formation that warrant attention. These include:
— the genetic structure and demography of a population such as degrees of inbreeding and effective population size (Templeton, 1981)
— the genetic control of diapause leading to allochronic isolation in association with host plant phenology (Tauber & Tauber, 1981)
— induction and other mechanisms of learning (Prokopy et al., p 123)
— the number of loci controlling host selection and survival abilities
— the taxonomic affinity of old and new host plants (Ehrlich & Raven, 1965; Bush, 1969) as well as their micro- and macro-abundance and distribution
— agricultural practices such as the widespread planting of monocultures and removal of defensive chemicals by selective breeding

In summary we conclude that in parasitic insects sympatric host race formation and speciation may occur frequently. But there is obviously a need for further empirical investigations and theoretical development that takes into account the unique biological qualities of host-parasite interactions.

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Food plants and speciation in the Papilio glaucus group

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Abstract

The range of the eastern tiger swallowtail, *Papilio glaucus*, covers over 4 billion hectares in North America, extending from southern Florida to the northern Canadian Yukon. Two different subspecies are recognized, with their zone of separation at 40-45°North latitude, which also corresponds to a plant transition zone. Differential foodplant utilization abilities and differences in diapause biology between the subspecies are described as partial explanations for the apparently sharp subspecies demarcation (or hybrid zone) in Wisconsin compared to New York State and New England. Preliminary studies involving hand-paired subspecies crosses are described which contribute to a better understanding of the genetic basis of known differences in diapause, colour, size, and foodplant detoxication abilities between the two subspecies, *P. glaucus glaucus* and *P. glaucus canadensis*.

Key-words: Papilio glaucus subspecies, diapause, evolution, geographic range, growth, speciation, wing patterns, genetics, food preferences

As a species, the eastern tiger swallowtail butterfly, *Papilio glaucus* L., is the most polyphagous of the world Papilionidae (Scriber, 1973). It occupies a geographic area wich encompasses the eastern half of the USA and nearly all of Canada (totalling over one billion hectares, Figure 1). Two subspecies are recognized, i.e., *Papilio glaucus glaucus* L. and *P. g. canadensis* Rothschild and Jordan, with their zone of separation at approximately 42°to 45°North latitude from Minnesota to New England (Remington, 1968; Scriber et al., 1982). Dramatic differences exist in the biology of these two subspecies, including different morphological and colour characteristics, different diapause biology (one univoltine, one multivoltine), different sizes, and different food plant detoxication and physiological processing abilities. While some research has been done with the western species group (*P. multicaudatus, P. rutulus*, and *P. eurymedon*; Brower, 1958, 1959a,b), little is known about the significance of these factors for reproductive isolation between the *Papilio glaucus* subspecies.

I will describe geographic differences in the biology and nutritional ecology of *Papilio glaucus* and present data from hand-paired subspecies crosses which contribute to a better understanding of the genetic basis of their differential abilities. Of
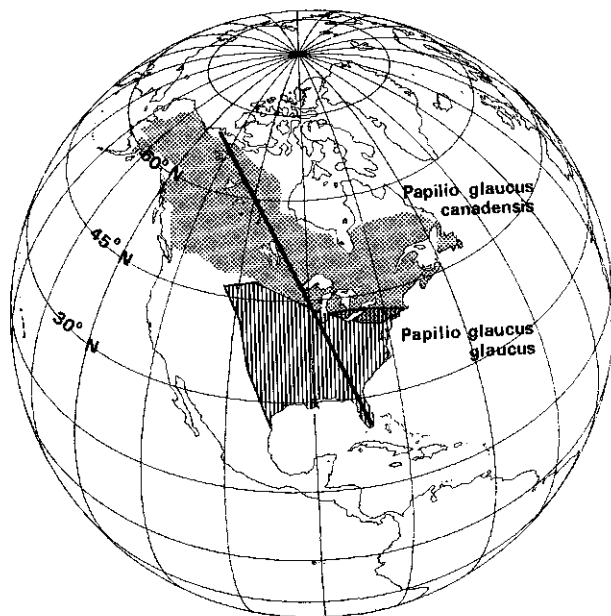
Fig. 1. The geographic range of the two subspecies of *Papilio glaucus*.

particular interest are the roles of food plants and diapause in the geographic delineation of the hybrid zone of interaction between *P. g. glaucus* and *P. g. canadensis*. Such knowledge of hybrid zones is vital in discrimination between allopatric and parapatric speciation (Endler, 1977; Atchley & Woodruff, 1981).

Foodplants

Across its vast geographic range, *Papilio glaucus* utilizes a variety of deciduous trees as food plants. Significant differences exist in larval survival and growth of different geographic populations of *P. g. glaucus* and *P. g. canadensis* on twelve different plants (Scriber et al., 1982; Scriber, 1982a). In addition, it has become apparent that certain of these food plants, e.g., *Liriodendron tulipifera* L. (a southern plant) and *Populus tremuloides* (a northern plant), may play a significant role in determining the geographic limits of each of the *P. glaucus* subspecies (Tables 1 and 2; Scriber, 1982b). It is also noteworthy that the potential food plant availability along a 6000-kilometer transect from southern Florida (25°N) to the northern Yukon (70°N) is greatest near the 40-45°N latitude at what might be considered a plant transition zone i.e., tropical and boreal species separate rather distinctly (Figure 1 and Table 3). It is likely that the close geographic correlation between the plant transition zone (Curtis, 1959) and the distributional limits of the two *P. glaucus* subspecies in Wisconsin (Figure 2) is not entirely due to host plant affiliations of local populations, however. In fact, black cherry *Prunus serotina* appears to

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Table 1. Larval survival to second instar on three key food plants. Initial numbers are indicated in parentheses.

Insect	Black cherry (*Prunus serotina*)	Tulip tree (*Lirio-dendron tulipifera*)	Quaking aspen (*Populus tremuloides*)
Papilio glaucus glaucus[1]			
with dark morph mothers	79% (43)	76% (67)	0% (64)
with yellow morph mothers	84% (61)	78% (76)	8% (80)
Papilio glaucus canadensis[2]	88% (73)	0% (88)	86% (80)
F$_1$ 'Hybrids' (*P.g.g.* × *P.g.c.*)[3]	77% (39)	82% (44)	73% (44)

1. *Papilio glaucus glaucus* from Pennsylvania (Schuylkill County, 1980) and Wisconsin (Richland County, 1979; Dane County, 1981).
2. *Papilio glaucus canadensis* from six counties on northern Wisconsin (1980) (Scriber et al., 1982).
3. Hand-paired in the laboratory in 1981.
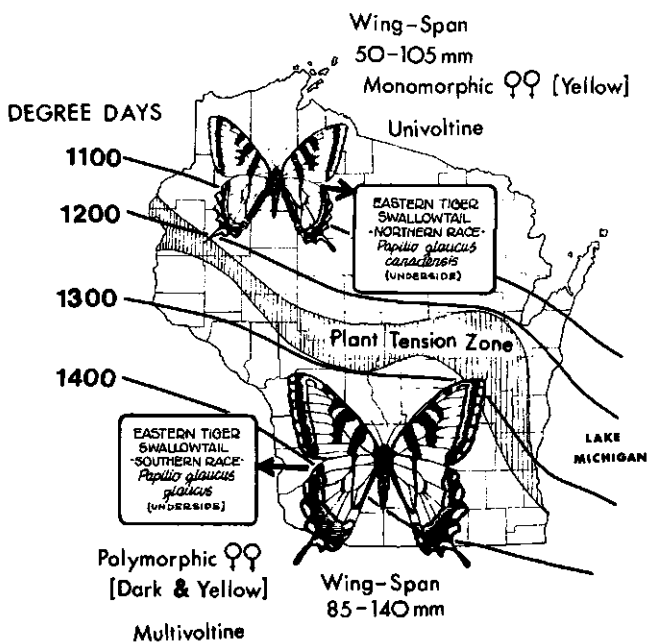
Fig. 2. Distributions of the two subspecies of *Papilio glaucus* in Wisconsin separate at the shaded area on the map, which represents the plant tension zone (Curtis, 1959; Scriber et al., 1982). Seasonal degree day accumulation (above 10°C) is based upon data from the Wisconsin Statistical Reporting Service (Madison; 20-year average). The plant tension zone basically represents the northernmost limit of multivoltinism potential in *P. glaucus*.

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Table 2. Total larval duration (days) on three key food plants. Data are presented as mean ± S.E.

Insect	Black cherry (*Prunus serotina*)	Tulip tree (*Lirio-dendron tulipifera*)	Quaking aspen (*Populus tremuloides*)
Papilio glaucus glaucus with dark morph mothers	31.2 ± 0.4 (13)[1]	28.6 ± 0.5 (29)	none survived
with yellow morph mothers	31.9 ± 1.0 (12)	27.5 ± 0.4 (36)	none survived
Papilio glaucus canadensis	32.0 ± 0.7 (40)	none survived	38.7 ± 0.8 (36)
F_1 'Hybrids' (*P.g.g.* × *P.g.c.*)	26.2 ± 0.6 (21)	26.6 ± 0.5 (21)	35.4 ± 0.9 (25)

1. Number of larvae.

be the favourite food plant for both subspecies throughout their range in Wisconsin (i.e., a middle plant in Table 3). Thus, food plant restriction (i.e. host races) is not the total explanation for why the two subspecies separate sharply in this narrow Wisconsin hybrid zone.

Diapause

Another explanation for the geographically stable hybrid zone in Wisconsin may involve the differential diapause biology of the two subspecies. The northern (*P. g. canadensis*) is believed to be everywhere univoltine, while the southern (*P. g. glaucus*) is multivoltine throughout its range. Using a conservative estimate of 11°C as the larval developmental threshold (Scriber & Lederhouse, 1982), it becomes obvious why a second brood of *P. g. glaucus* is virtually impossible north of the plant tension zone in Wisconsin, e.g., on the best food plant in the early season at the peak of leaf quality, 600 degree days are required from egg to adult emergence (Figure 2). However, while this might partially explain the northern limits of multivoltine *P. g. glaucus*, it does not explain why *P. g. canadensis* does not occur south of 41-42°N latitude. Since many acceptable and suitable food plants for *P. g. canadensis* exist throughout the range of *P. g. glaucus* (Scriber et al., 1982), it remains uncertain why *P. g. canadensis* appears restricted in its southern distribution limits.

Hybrids

A variety of evidence supports the concept of a hybrid zone of interaction between these subspecies, however the actual degree of interbreeding and the various potential mechanisms of reproductive isolation have not yet been investigated. Sev-

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Table 3. Potential food plants at various latitudes for *Papilio glaucus* along the 6000-km transect from southern Florida to the Canadian Yukon (Figure 1).

Latitude (°N)	Plants species available			
	northern[1]	middle[2]	southern[3]	total
70—	0			0
—65	2			2
65—	2			2
—60	2			2
60—	3			3
—55	4			4
55—	5			5
—50	7			7
50—	8	3		11
—45	8	6		14
45—	7	6		13
—40	2	6	4	12
40—		6	5	11
—35		6	4	10
35—		4	6	10
—30		4	5	9
30—		3	4	7
—25			1	1

1. The northern species include quaking aspen, paper birch, balsam poplar, pin cherry, choke cherry, mountain ash (2 spp.) and yellow birch.
2. The middle species include big-toothed aspen, basswood, green ash, black cherry, American hornbeam, and white ash.
3. The southern species include common hoptree, sassafras, spicebush, tulip tree, mountain magnolia, and sweet bay (Rutaceae, Lauraceae and Magnoliaceae).

eral morphologically distinguishable traits are suspected to differ between the two subspecies (Scriber, 1982a), one of which is depicted in Figure 3. Hand-paired subspecies crosses support this contention and provide a reference base which suggests that hybridization is not only possible, but is extremely likely where these subspecies adjoin. This hybrid zone appears to be much broader geographically in New York State than in Michigan and Wisconsin (Figure 1; Scriber et al., 1982). For example, the ventral forewing yellow band and wide hind wing black band (Figure 2), the voltinism patterns, and the lack of dark morph females in Central New York are strongly 'canadensis' in nature, however these populations naturally utilize tulip tree (*Liriodendron tulipifera*) with excellent success, unlike females of *P. g. canadensis* from northern Wisconsin (Scriber & Feeny, 1979; Scriber et al., 1982).

The above-mentioned field observations regarding apparent subspecies 'hybrids' are consistent with laboratory data from hand-paired subspecies F_1 hybrids. The F_1

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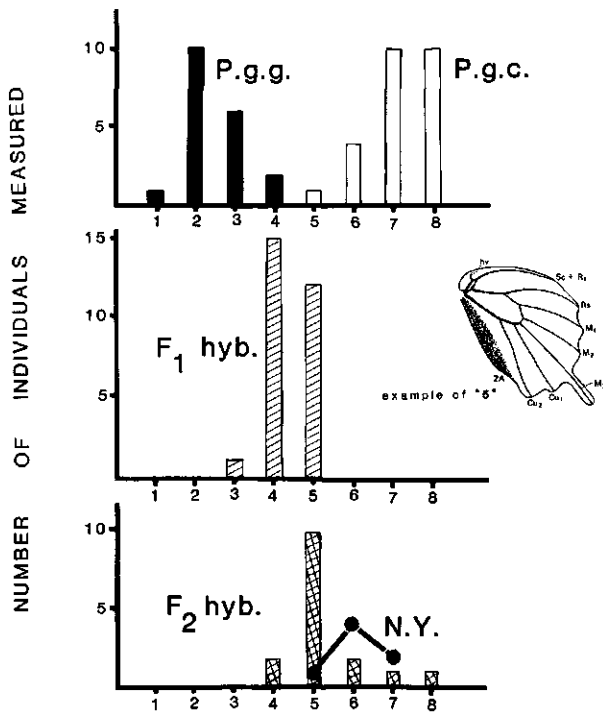
Fig. 3. Proportional distance of the ventral black band in the anal wing cell to the origin of the second cubital branch vein in males of the *Papilio glaucus glaucus (P.g.g.)* and *P. g. cana-densis (P.g.c.)*, F_1 hybrids, F_2 offspring and a natural population from central New York state (N.Y.).

hybrid larvae are able to survive (Table 1) and grow (Table 2) on plants which are generally toxic on one or the other of the pure subspecies. This might explain the ability of central New York populations to survive and grow on both *Populus trem-uloides* and *Liriodendron tulipifera.* Assessing the proportion of hybrids in these con-tact zones may be possible with multivariate analyses, especially since some traits are known to be intermediate in laboratory hybrids (Figure 3). While size difference in adults have been used in classifying *P. glaucus* subspecies (Tyler, 1975; Figure 2), and while there is a genetic basis to these differences (Scriber et al., 1982), the suit-ability of the food plant species used by the larvae can totally mask any such size differences. For example, an analysis of wing length of the F_1 hybrid adults reveals the importance of food plants in size determination of *Papilio glaucus* (Figure 4). The adult wing span of these hybrids nearly spans the entire range of sizes for both subspecies (equivalent of 70-130 mm; Figure 2).

Understanding the genetic basis and heritability of known differences in dia-

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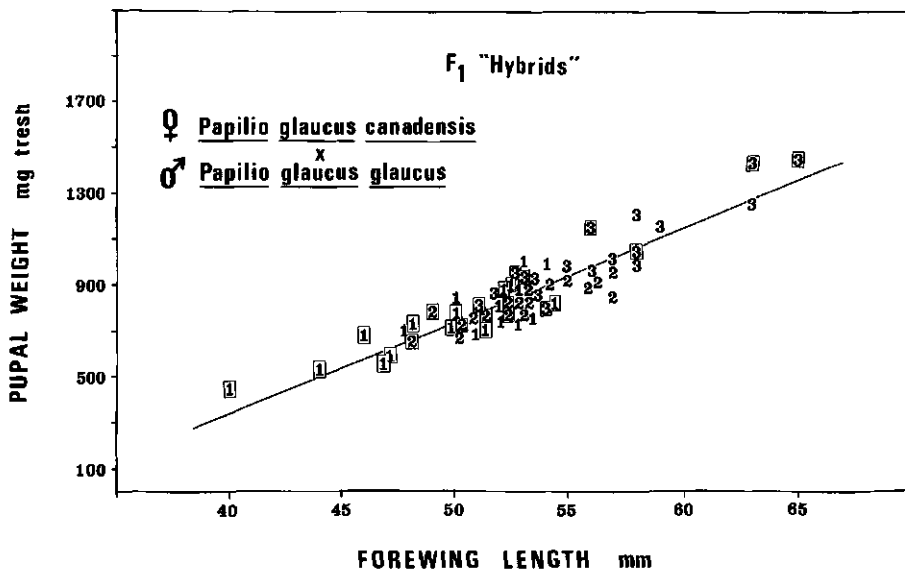
Fig. 4. The relationship between pupal weight and wing length in F_2 'Hybrids' of *Papilio glaucus glaucus* (male) × *P. g. canadensis* (female) as a function of larval food plant. 1: *Populus tremuloides*; 2: *Liriodendron tulipifera*; 3: *Prunus serotina*.

pause, colour, size, food plant choices and detoxication abilities should help clarify the evolutionary relationships between *Papilio glaucus* subspecies and perhaps also provide new insights regarding the relationships with their three closely related species in western North America. In the process of more accurately determining the genetic, behavioural, and physiological mechanisms of ecological adaptation, the actual role of food plants in the speciation process may become more clearly evident.

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Geographic variation and host plant adaptation of the Colorado potato beetle

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Abstract

Differences in ecophysiological and genetic traits among selected beetle populations from North America and continental Europe were investigated. Rearing data revealed no significant change in host plant specificity among beetle populations from natural and cultivated hosts. Host plant adaption was evident, however, in an Arizona beetle population and a laboratory strain which exhibit higher tolerance to certain otherwise unsuitable plants. Crossbreedings showed that the trait for host adaptation is heritable. Adult diapause is induced by photoperiod or by adverse host conditions. These two strategies of seasonal adaptation provide synchronization for the species to adapt to host plants in diverse geographic regions. Genetic divergence was revealed by cytogenetic analysis of chromosomal karyotypes and gel electrophoresis of isozymes. These findings imply that the Colorado potato beetle is highly adaptable and capable of further expanding its host range and geographic distribution.

Key-words: Leptinotarsa decemlineata, geographic variation, insect populations, isozymes, diapause, food preferences, biotypes, genetics, evolution

Analysis of insect host-plant relationships has been traditionally focused on interactions between the two biological systems. Much less is known about the intrinsic variability of each biological system and how such factors influence insect-plant relationships. Since insects and plants are dynamic systems subject to spatial and temporal changes, some variations in the biological characteristics of each system are to be expected. Investigations to define such variations among populations are crucial to the understanding of the ecological and evolutionary relationships between insects and plants. Agricultural insect pests that are cosmopolitan in distribution are well suited for investigation of such population variations. The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is an ideal candidate because of its wide geographic distribution in North America and continental Europe. Several previous studies have examined the ecophysiological variations among geographic populations in North America (Hsiao, 1978; 1982) and geographic diversity among beetle populations in Europe (de Wilde & Hsiao, 1982). This paper describes current findings on host plant adaptations and genetic variability among geographic populations.

Pattern of host plant adaptation among geographic populations

Evidence for host plant adaptation was first noted among four beetle populations from southwestern United States (Hsiao, 1978). The natural hosts for these four populations were *Solanum rostratum* for the New Mexico and Texas beetles; *S. elaeagnifolium* for the Arizona beetles; and the cultivated potato, *S. tuberosum*, for the Utah beetles. The possibility that host plant adaptation may exist in other geographic regions, especially among populations that infest cultivated hosts, was investigated with six beetle populations from Europe (de Wilde & Hsiao, 1982; Hsiao, 1982). These populations were from Wageningen, the Netherlands; St. Clair, France; and Granada, Spain in western Europe and Poznan, Poland; Budapest, Hungary; and Athens, Greece in eastern Europe. A laboratory strain maintained for 25 years at Wageningen, the Netherlands was also included in the study because of its unusual adaptability to a variety of hosts. The European beetle populations were well suited for the study because they have been separated from the North American stock for nearly 60 years and have subsisted entirely on cultivated hosts, mainly potato and eggplant, and occasionally on tomato. The growth performance of these European beetles was evaluated on 11 solanaceous plants i.e., *S. tuberosum, S. chacoense, S. dulcamara, S. elaeagnifolium, S. melongena, S. rostratum, Lycopersicum esculentum, S. nigrum, S. luteum* and two varieties of *Hyoscyamus niger*.

Recently, a beetle population from Yautepec, Morelos, Mexico was studied because it represents the most southerly distribution of the species. In its natural habitats it feeds on *S. rostratum* and *S. angustifolium*. The host plant affinity was determined with laboratory rearings from hatching to adult emergence on nine solanaceous plants at 25°C and a 16-hour photophase, a rearing condition similar to that used for evaluation of other geographic populations. The Mexican beetles were unable to develop beyond the second larval instar on the following five plants: *S. nigrum, S. elaeagnifolium, S. sarachoides, S. carolinense* and *L. esculentum*. It developed well, however, on four plants: *S. rostratum, S. tuberosum, S. melongena* and *S. dulcamara* (Table 1). The surprisingly high rearing success on these plants proves that the feeding requirements of the Mexican beetles are similar to those of other geographic populations. On the other hand, their inability to feed on five of the

Table 1. Growth performance of a Colorado potato beetle population from Yautepec, Morelos, Mexico on four species of *Solanum*. Rearings held at 25°C and 16-hour photophase.

Host plant	Number of insects	Mortality (%)		Number and mean pupal weight ± S.D.(mg)		Development duration ± S.D. (days)	Adult emergence (%)
		larvae 1-4th	prepupae and pupae	female (n)	male (n)		
S. tuberosum	159	33.3	6.3	(51) 98.1± 9.9	(45) 85.5±8.0	19.6±1.1	60.4
S. rostratum	111	32.4	15.3	(28) 84.8±10.6	(30) 73.5±7.2	19.5±1.2	52.3
S. dulcamara	91	51.6	1.1	(18) 100.0±10.6	(25) 89.1±9.3	19.7±1.3	47.3
S. melongena	115	35.6	9.6	(22) 86.8±10.3	(41) 76.2±9.1	20.8±1.2	54.8

tested plant species clearly demonstrated that the Mexican beetle population has a more restricted host range than that of other populations.

On the basis of the rearing data from these 12 beetle populations, the pattern of variations in host plant adaptation can be assessed. To facilitate a direct comparison, these 12 populations are divided into three groups based on their host associations. The first group includes four North American beetle populations that feed on natural hosts. The second group consists of the Utah population and the six European beetle populations that feed on cultivated hosts. The laboratory strain is a third group because of its unique adaptability to rearing conditions. Figure 1 shows that the rearing success of beetle populations from the natural and cultivated hosts does not differ significantly on five of the hosts, i.e., *S. tuberosum*, *S. dulcamara*, *S. rostratum*, *S. melongena* and *H. niger*. The wide acceptance of these plants by the various beetle populations supports the view that the original host shift of the Colorado potato beetle from *S. rostratum* to the cultivated potato does not require adaptive changes in the insect. With *S. elaeagnifolium*, marked differences in rearing success were found between populations from the natural and cultivated hosts (Figure 1). Figure 2 compares the survivorship curves of 11 populations reared on *S. elaeagnifolium*. The Arizona beetles are the only field population that survives well on this plant. The New Mexico beetles show some degree of adaptation, with a 35% rearing success. Among beetle populations from cultivated hosts, survival is low, with only about 10% rearing success. Mortality occurs throughout the four larval instars, indicating that the presence of deterrents is the cause of low adaptation to this plant. The high tolerance of the Arizona beetles and the laboratory strain to the deterrent enables them to survive well on *S. elaeagnifolium*. This finding demonstrates conclusively that adaptation to *S. elaeagnifolium* requires considerable physiological adjustment by the Colorado potato beetle.

The laboratory strain showed consistently high rearing success on a variety of plants (Figures 1 and 2; de Wilde & Hsiao, 1982). The unique adaptability of this strain is probably the consequence of rearing practices. In the maintenance of insect cultures, it is not uncommon to select individuals that grow uniformly and vigorously. Moreover, the diapausing adults were sacrificed for physiological experiments. The cumulative effects of such practices over nearly 25 years have unintentionally produced a highly adaptable laboratory strain of the Colorado potato beetle. Although the laboratory strain has been maintained exclusively on the cultivated potato, it is remarkably adaptable to other natural and cultivated hosts. In addition to high rearing success on these hosts, it develops more rapidly and produces larger progeny than any field populations (Table 2; Hsiao, unpubl.). Furthermore, the females are more fecund and have a shorter preoviposition time than that of field populations. Clearly, the laboratory strain has developed a higher degree of tolerance to deterrents existing in various solanaceous plants. The considerable differences in adaptability to host plants between the laboratory strain and field populations are indicative of the capability of the Colorado potato beetle to further expand its host range.
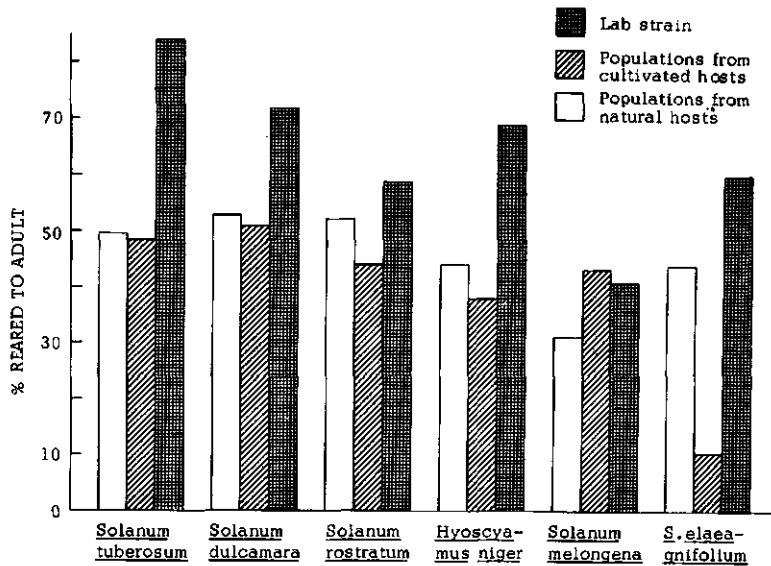
Fig. 1. Comparison of rearing success of Colorado potato beetle populations from natural and cultivated hosts and a laboratory strain on six hosts.
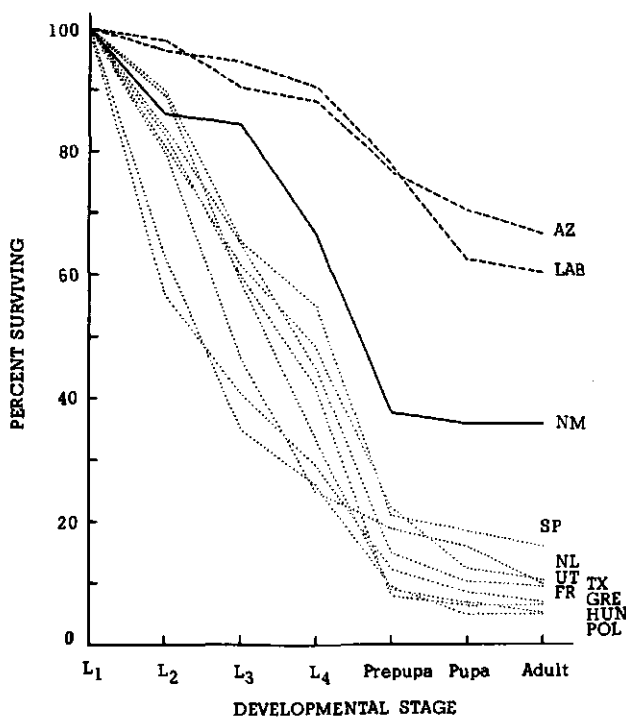
Fig. 2. Survivorship curves of 11 geographic populations of the Colorado potato beetle reared on *Solanum elaeagnifolium* (see text for explanation of abbreviations).

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Table 2. Growth performance on *Solanum tuberosum* of two Colorado potato beetle populations and their reciprocal crosses.

Population	Number of insects	Mortality (%)		Number and mean pupal weight ± S.D.(mg)		Development duration ± S.D. (days)	Adult emergence (%)
		larvae 1-4th	prepupae and pupae	female (n)	male (n)		
Parent generations							
Mexico,							
Morelos	159	33.3	6.3	(51) 98.1 ± 9.9 (45)	85.5 ± 8.0	19.6 ± 1.1	60.4
Lab strain	95	6.3	9.5	(39) 152.3 ± 15.1 (41)	134.8 ± 13.6	18.5 ± 0.8	84.2
Crosses							
Lab strain ♀ × Mexico ♂							
F₁ progeny	154	3.9	5.2	(66) 145.6 ± 18.3 (74)	133.3 ± 14.8	17.9 ± 0.8	90.9
F₂ progreny	149	16.1	7.4	(55) 131.4 ± 11.8 (59)	108.8 ± 10.9	18.6 ± 1.0	76.5
Mexico ♀ × Lab strain ♂							
F₁ progeny	87	0	5.7	(41) 124.8 ± 14.5 (41)	96.0 ± 17.2	18.4 ± 1.3	94.3
F₂ progeny	142	32.4	10.6	(42) 127.9 ± 16.4 (39)	112.0 ± 16.2	19.5 ± 1.3	57.0

Hybridization among geographic populations

Geographic populations were crossbred to determine compatibility and the basis for host plant adaptation. These experiments are crucial because of the lack of such biological information and the confusion that has existed concerning the taxonomic status of the species. Populations from Utah, Arizona and Texas have been successfully crossbred (Hsiao, 1982). Recent hybridization experiments focus on the beetle population from Morelos, Mexico, which has previously been regarded as a separate species, *Leptinotarsa multitaeniata*, because of its southerly distribution and slight morphological variations from other geographic populations. The Mexican beetle was crossbred with the laboratory strain, and their progeny were reared on potatoes at 25°C and a 16-hour photophase. Table 2 summarizes the results of rearing for two generations. The most striking feature of these data is the hybrid vigour of the F₁ progeny. The rearing success of the two reciprocal crosses is 90.9 and 94.3%, compared with 84.2% for the laboratory strain parent and 60.4% for the Mexican parent. The F₁ hybrids also developed more rapidly and attained larger pupal size than did their parents. In the F₂ generations, the hybrids show an intermediate growth performance compared to the parent generations. It should be pointed out that the size of the beetles of the Mexican population and the laboratory strain represents the two extremes found among various geographic populations. The fact that these two populations interbreed readily proves that they are the same species.

The unique adaptiveness of the Arizona beetle population to *S. elaeagnifolium* raises the question as to whether the trait for such host adaptation is heritable. Laboratory crosses were made between Arizona and Utah beetle populations to determine fitness with regard to survival on two host plants, *S. tuberosum* and *S. elaeag-*

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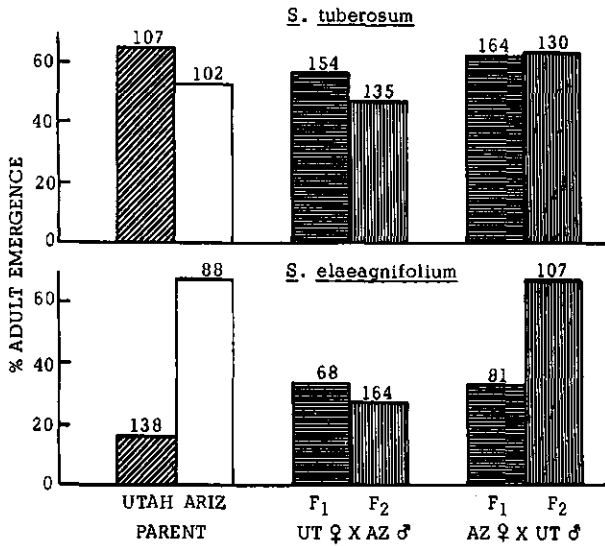
Fig. 3. Rearing success of two geographic populations of the Colorado potato beetle and their progeny on two plants. The number at the top of each column indicates the number of newly-hatched larvae used in each experiment.

nifolium (Figure 3). In the parent generations, the Utah beetles had a very low (16%) survival rate on *S. elaeagnifolium*, whereas the Arizona beetles had a very high (68%) survival rate. On *S. tuberosum*, the Utah beetles had a higher (65.4%) rearing success than the Arizona beetles (53.0%). In the F_1 generation, the two reciprocal crosses showed similar survivorship (33%) on *S. elaeagnifolium*; on *S. tuberosum* they survived as well as did the parents. Hybrid vigour was not apparent in either cross. In the F_2 generation, the crosses between the Arizona female and the Utah male beetles survived remarkably well on *S. elaeagnifolium* (68.2%), producing twice the percentage of adults of the F_1 generation and equaling the rearing success of the Arizona parent generation. In contrast, the F_2 generation from crosses between the Utah female and the Arizona male beetles had a low percentage of rearing success on *S. elaeagnifolium* (28%) compared to the F_1 generation (33.8%), but was still higher than that of the Utah parent generation. The rearing success on *S. tuberosum* was slightly reduced in the F_2 generation (47.7%) compared to the F_1 generation (57.1%) and the Utah parents (65.4%). These results clearly demonstrate that the rearing success of the progeny is dependent upon the combination of the parents. The fact that the F_2 hybrids of the cross between the Arizona female and the Utah male beetles survived best on *S. elaeagnifolium* indicates that the adaptation to this plant is inherited mostly through the female parent. The trait for host adaptation is not sex-linked, however, since progeny of the Arizona male and Utah female beetles also had some degree of improvement in feeding on *S. elaeagnifolium* compared to the Utah parents. Since the trait for host adaptation is neither dominant nor recessive, a polygenic inheritance is likely to be involved in the adaptation of the Arizona beetles on *S. elaeagnifolium*.

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Seasonal adaptation of various geographic populations

Photoperiodic induction of adult diapause has been investigated with 12 geographic populations of the Colorado potato beetle. On the whole, populations from Europe and North America show a predictable pattern of response, with a gradual increase in the critical photophase as the species are distributed northward (Hsiao, 1982; de Wilde & Hsiao, 1982). Such responses reflect a seasonal adaptation well synchronized with the availability of host plants. The population from Roma-Los Saenz, Texas (26°24'N) was the only exception; it did not respond to photoperiod. Adults reared at photophases between 10 and 18 h did not enter diapause but continued to reproduce. Recent unpublished rearing data also showed that the beetle population from Yautepec, Morelos, Mexico (18°52'N) is insensitive to photoperiod i.e., it continued to reproduce at photophases between 10 and 16 h. These results indicate that two mechanisms are involved in the induction of adult diapause of the Colorado potato beetle under natural conditions. Laboratory studies have shown that besides photoperiod, adverse host plant conditions and starvation can induce adult diapause (de Wilde et al., 1969). Beetles fed with physiologically aged potato leaves ceased to reproduce and diapause followed. Such a mechanism is likely to be responsible for inducing adult diapause in beetle populations that feed on native hosts in southwestern United States and in Mexico. Field observations showed that in these arid regions the host plants are suitable for development and reproduction of the Colorado potato beetle within a brief period after summer rains. Consequently, beetle populations in these southern habitats can produce only one or at most two generations a year, even though other environmental conditions such as temperature may be adequate for continual development and reproduction. By evolving two strategies of seasonal adaptation, the Colorado potato beetle is able to exploit various food plants in vastly differing geographic regions. Since the trait for photoperiodic induction of adult diapause is under genetic control, as demonstrated by crossbreedings between the Texas and Utah beetles (Hsiao, 1982), it would be extremely interesting to determine whether these two modes of seasonal adaptation serve as an effective isolating mechanism in restricting gene flows among geographic populations.

Genetic variation among geographic populations

Genetic variability among beetle populations was investigated to obtain information that can be used to explain host plant adaptation and seasonal adaptation. Cytogenetic analysis of chromosomal karyotypes has yielded significant results. Most important was the discovery of a pericentric inversion on the No. 2 chromosomes of the Colorado potato beetle (Hsiao & Hsiao, 1980). Hybridization experiments showed that the pericentric inversion is a stable chromosomal mutation and follows Mendelian inheritance (Hsiao & Hsiao, 1982). All three karyotypes, the metacentric, the acrocentric and the heterozygote, are found in North American beetle populations (Table 3 and unpublished data). The metacentric race is the original karyotype and is found in the Mexican, Arizona and Utah (Kanab) beetle populations. The acrocentric race is derived from the metacentric race and occurs in

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Table 3. Genetic variability among geographic populations of the Colorado potato beetle.

Population	Host plant (Solanum)	Karyotype of No. 2 chromosomes	Polymorphic loci[1]	Mean heterozygosity ± S.D.[1]
Washington, Yakima	*S. tuberosum*	heteromorphic	0.455	0.196 ± 0.071
Utah, Logan	*S. tuberosum*	heteromorphic	0.636	0.195 ± 0.078
Utah, Kanab	*S. tuberosum*	metacentric	0.545	0.227 ± 0.079
Texas, Eden	*S. rostratum*	heteromorphic	0.636	0.227 ± 0.083
Oklahoma, Bixby	*S. melongena*	acrocentric	0.455	0.227 ± 0.082
Michigan, Montcalm Co.	*S. tuberosum*	acrocentric	0.545	0.232 ± 0.081
Virginia, Montgomery Co.	*S. tuberosum*	acrocentric	0.455	0.225 ± 0.083
New Jersey, Dayton	*S. tuberosum*	acrocentric	0.455	0.205 ± 0.081
Florida, Hillsborough Co.	*S. tuberosum*	acrocentric	0.545	0.220 ± 0.079
Spain, Sevilla, Las Cabezas	*S. tuberosum*	acrocentric	0.455	0.196 ± 0.092
Laboratory strain	*S. tuberosum*	acrocentric	0.545	0.235 ± 0.077
Mexico, Morelos, Yautepec	*S. angustifolium*	metacentric	0.455	0.092 ± 0.043

1. Allele frequencies of 11 isozyme loci.

beetle populations from central and eastern United States. The heteromorphic race includes all three karyotypes in the same population and is found in beetle populations from western United States. The cytogenetic data reveal not only karyotype variability but that a considerable amount of gene mixing is occurring in geographic populations. No correlation can be established, however, between chromosomal karyotype polymorphism and host plant affinity of various beetle populations.

A recently completed study compares isozyme variations among 12 beetle populations (Jacobson & Hsiao, 1982). Of the 11 genetic loci examined by starch gel electrophoresis, four are monomorphic (fumarase, glutamate oxoalacetate transaminase, superoxide dismutase and α-glycerophosphate dehydrogenase) and seven are polymorphic (malic dehydrogenase, malic enzyme, acid phosphatase, esterase I, esterase II, glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase). The superoxide dismutase was a diagnostic locus distinguishing the Mexican beetle population from 11 other populations. With the exception of the Mexican population, which had a low value for heterozygosity, no significant difference in the proportion of polymorphic loci or heterozygosity was found among geographic populations (Table 3). The mean heterozygosity of all populations examined was 0.206 ± 0.039, which is slightly higher than the value (0.151) for other groups of insects, but is well within the range reported for other species of Coleoptera. The 11 populations from the United States and Europe were similar genetically, with an average genetic distance of 0.022 ± 0.013 and a fixation index of 0.068. The fixation index infers that 93.2% of the total variation is found within populations, and only 6.8% is ascribable to genetic differences among populations. The genetic distance between the Mexico population and the others was 0.212 ± 0.026, which is comparable to values reported for subspecies or semispecies of *Drosophila*. The isozyme data when combined with previous hybridization results (Table 2) provide convincing proof that two subspecies of the Colorado potato beetle exist. The high heterozygosity found among

beetle populations is further proof that there is considerable variability in the species. This finding implies that the Colorado potato beetle has high potential for adapting to novel environments.

Conclusion

The Colorado potato beetle is a specialized feeder, with its host range restricted mainly to a dozen plant species of the genus *Solanum*. Yet, this beetle has been very successful in exploiting a variety of native and cultivated solanaceous plants in a wide range of habitats. The existence of different patterns of host plant utilization raised the question of how much variability in host plant adaptation exists or is developing among geographic populations. Extensive laboratory rearings of beetles from various hosts and geographic regions revealed that the degree of host specificity has not changed significantly, as evidenced by the rearing success on five hosts: *S. tuberosum*, *S. dulcamara*, *S. rostratum*, *S. melongena* and *H. niger*. An Arizona beetle population was, however, an exception, it develops and reproduces well on *S. elaeagnifolium*, a plant unsuitable for other beetle populations. Crossbreeding experiments showed that the trait for this unique host plant adaptation is heritable. The overall higher rearing success of the laboratory strain on various host plants as compared to field populations suggests that the potential for developing adaption to new hosts exists in this species.

Two strategies of seasonal adaptation have evolved in the Colorado potato beetle. In Mexico and southern Texas, adult diapause is probably induced by adverse host plant conditions. In more northerly habitats, adult diapause is regulated by photoperiod and is under genetic control. Both seasonal adaptation strategies provide synchronization that is necessary for the species to exploit host plants in diverse geographic regions. In other words, seasonal adaptation is an essential part of the overall host plant adaptation strategy of the Colorado potato beetle.

Cytogenetic data revealed polymorphism in chromosomal karyotypes among beetle populations in North America. Isozyme data provided further proof that there is considerable genetic variability in the species. The high heterozygosity found among geographic populations implies that the Colorado potato beetle has high potential for adapting to novel environments. When data from ecophysiological and genetic analyses are combined, it is evident that the Colorado potato beetle is highly adaptable and capable of further expanding its host range and geographic distribution.

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The herbivores of Passiflora: comparison of monophyletic and polyphyletic feeding guilds

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Abstract

Two types of insects commonly attack the new shoots of Central American *Passiflora* vines. These are *Heliconius* butterflies and flea beetles. All *Heliconius* attack Passifloraceae, and they constitute a monophyletic feeding guild; i.e. a guild which is descended from a common ancestor, probably ancient, which also fed on Passifloraceae. In contrast, the *Passiflora*-feeding flea beetles form a polyphyletic feeding guild, in which beetles in different genera independently (and more recently?) evolved a preference for *Passiflora*. The host plant preferences in these two guilds were compared, and were found to be remarkably similar except for a tendency for the butterflies to be more host-specific. Both guilds had two generalist species, as well as one specialist which fed on *P.* subgenus *Granadilla* hosts, and four specialists which fed on *P.* subgenus *Plectostemma* species. Both guilds had highly similar diversity indices in two habitats, and both guilds responded similarly in feeding trials using *P.* subgenus *Plectostemma* and *P.* subgenus *Granadilla* hosts. These results indicate that the superficial pattern of feeding preferences and species diversity was not strongly influenced by the type of ancestry in the insect-plant association, but was instead determined by characteristics of the host plants.

Key-words: evolution, Chrysomelidae-Halticinae, *Heliconius* species, *Passiflora* species, food preferences, generalists, insect diversity, plant diversity, specialists, speciation

Ehrlich & Raven (1964) proposed that evolving insect-plant associations have been a major force in the generation of diversity in plant and animal communities. Although this idea was very influential in stimulating interest in coevolutionary processes and insect-plant interactions (Gilbert & Raven, 1975), it has proven to be very difficult to test. For example, at the present time there is very little evidence that groups of insects such as the butterflies have strongly affected plant diversification, as Jermy (1976) and Janzen (1981) have recently discussed. Given the difficulty of testing the Ehrlich & Raven hypothesis, it may be of interest to investigate the related hypothesis that a guild (i.e. functionally related group; Root, 1967) feeding on a taxon of plants will be more diverse if they have had a long evolutionary association with that group of plants. This result is to be expected if the insects evolve greater specialization in host plant use, which would then allow more diversification among the insects in the guild. This hypothesis may be tested by compar-

ing guilds of insects which vary in the degree of evolutionary association with their host plants, and observing whether the guilds with the closer evolutionary associations are more specialized and/or more diverse. Because host plant diversity, abundance, and geographic distribution also affect herbivore diversity (Gilbert & Smiley, 1978), it is desirable to eliminate these effects by comparing guilds which feed on the same host plants. In this paper I compare two such feeding guilds which both consume the new shoots and leaves of *Passiflora* (Passifloraceae).

Central American *Passiflora* species are eaten by several types of herbivorous insects (Gilbert, 1977). At the La Selva Field Station of the Organization for Tropical Studies (10°25'N, 84°0'W), two guilds of insects feed upon the new shoots and new leaves. These are the *Heliconius* butterflies (Lepidoptera: Nymphalidae) and several species of flea beetles (Coleoptera: Chrysomelidae, Alticini). The *Heliconius* guild is monophyletic, that is, it is derived from a common ancestor which also fed on Passifloraceae; in fact the entire tribe Heliconiini probably derived from a Passifloraceae-feeding ancestor (Ehrlich & Raven, 1964; Benson et al., 1976). In contrast, the flea beetle guild is polyphyletic, i.e., the flea beetles have independently and probably more recently evolved a preference for *Passiflora* at least four times, as evidenced by the fact that each of the four identified *Passiflora*-feeding genera are known to feed on other families of plants than Passifloraceae. For example, the *Altica* species which, like the other *Passiflora* feeders, appears to be a *Passiflora* specialist (Smiley, unpublished observations), is a member of a large genus which is known to feed upon 18 plant families in the Northeastern United States alone (Wilcox, 1979). The other flea beetle genera I have collected from *Passiflora* are also known from many plant families: *Disonycha*, at least ten families including monocots and gymnosperms; *Monomacra*, on Phytolaccaceae as well as Passifloraceae; and *Strabala*, on five families including Gramineae and Leguminosae (Wilcox, 1979; Blake, 1953). This pattern indicates that the flea beetles have had a much looser evolutionary association with the *Passiflora* than the *Heliconius*, and by comparing the two groups it may be possible to observe the effects of such differences. Of course, observed differences could be due to differences in life-history between butterflies and beetles. Nevertheless, such comparisons may at least set an upper limit as to the importance of long-term evolutionary associations. If the two guilds were found to be nearly identical in diversity and host specialization it would be a strong argument that long-term evolutionary association has had little direct influence on the generation of insect diversity in the *Heliconius*.

Materials and methods

To compare these guilds I collected three types of data from the field site at La Selva. Data on host plant use were obtained in all months of the year between March 1975 and December 1979 by collecting *Heliconius* eggs, larvae, and flea beetle adults from approximately 250 *Passiflora* vines belonging to 14 species in 4 subgenera (Smiley, 1978a). These plants were all well-mixed among each other, not occurring in patches, such that an insect travelling from one plant to another of the same species would be highly likely to encounter several other species on the way. Larval flea beetles were uncommon and appeared to cause less herbivore damage

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Table 1. *Heliconius* eggs and flea beetle adults collected on *Passiflora* at La Selva. A: *Passiflora* subgenus *Astrophaea*; D: *P.* subgenus *Distephana*; G: *P.* subgenus *Granadilla*; P: *P.* subgenus *Plectostemma*; PIT: *P. pittieri*; VIT: *P. vitifolia*; AMB: *P. ambigua*; QUAD: *P. quadrangularis*; OER: *P. oerstedii*; LOB: *P. lobata*; COR: *P. coriacea*; AUR: *P. auriculata*; BI: *P. biflora* COST: *P. costaricensis*.

Herbivore species	Number of plants[2]	*Passiflora* species									
		A	D	G		P					
		PIT	VIT	AMB	QUAD	OER	LOB	COR	AUR	BI	COST
Number of eggs											
Heliconius cydno		24	9	4	2	13	0	2	6	4	0
H. hecale		0	15	0	0	3	0	0	1	0	0
H. melpomene		0	0	0	0	10	0	0	0	0	0
H. ismenius		0	0	1	0	0	0	0	0	0	0
H. charitonia		0	0	0	0	0	5	0	0	0	0
H. erato		0	0	0	0	0	0	0	0	13	0
H. hecalesia		0	0	0	0	0	0	0	0	8	0
H. sara[1]		0	0	0	0	0	0	0	16	0	0
H. sapho[1]		1	0	0	0	0	0	0	0	0	0
H. doris		0	0	0	0	0	0	0	0	0	0
Number of flea beetle adults											
Altica sp.	20	1	0	1	0	6	33	0	8	17	3
Monomacra sp.	19	0	6	0	7	17	3	0	4	2	1
Strabala sp. 1	10	0	2	19	0	5	0	0	0	0	0
Disonycha sp.	3	0	0	0	0	0	0	0	1	4	0
Alticini sp. 1	8	0	0	0	0	0	0	0	4	4	0
Strabala sp. 2	7	0	0	1	0	0	12	0	0	0	0
Alticini sp. 2	5	0	0	0	0	0	4	0	0	3	0
Alticini sp. 3	1	0	0	0	0	0	0	0	0	0	1
Alticini sp. 4	1	0	0	0	0	0	1	0	0	0	0

1. Egg masses collected.
2. Number of different individual plants from which flea beetles were collected.

than the adults. Flea beetles were identified to the lowest taxonomic level possible by the staff of the U.S. National Museum. These data on host plant use are presented in Table 1.

Data on guild diversity were collected during the same time period in primary forest, secondary forest, forest edge, and a 2- to 3-hectare artificial clearing. In these habitats the *Passiflora* plants were counted, *Heliconius* adults were captured with a net, and flea beetle adults were collected, so that relative abundances and therefore diversity indices could be calculated, as shown in Table 2.

Feeding trials, the third type of data collected, were done so as to compare patterns of feeding specialization in the two guilds. *Heliconius* feeding trials were conducted by rearing the larvae from hatching to pupation as described in Smiley (1978b), while flea beetle feeding trials were performed by confining the beetles in small cages with circular wire screen sides approximately 20 cm high and clear plas-

Table 2. Diversity of *Heliconius*, flea beetles, and *Passiflora* host plants. Diversity index (e^H; Hill, 1973) calculated for *Heliconius* captured, flea beetles collected, and *Passiflora* tagged and counted in three habitats at La Selva. Original data from Smiley (1978b) and Table 1.

Habitat	*Heliconius*	Flea beetles	*Passiflora*
Successional plots	3.46	3.36	2.96
Secondary forest and forest edge	5.88	5.50	6.91
Primary forest	1.54	4.56	5.46
All habitats	5.36	5.53	9.45

tic dishes for the top and bottom. Host plant cuttings with stems immersed in water were introduced into the cage for 2 days with wild-caught beetles, and the amount of feeding recorded. Three species of beetle were tested in this way (Table 3), along with six species of *Heliconius*.

Comparison of feeding guilds

Comparison of the two guilds in Table 1 indicates that *Heliconius* appear to be more specialized in terms of host plant use than the flea beetles, since 5 out of 7 species were found to use one species of host as opposed to 0 out of 7 for the flea beetles (ignoring species with fewer than 5 host records). However, aside from this

Table 3. Herbivore feeding trials on *Passiflora* foliage. Results of feeding trials using newly hatched *Heliconius* larvae and adult flea beetles on La Selva *Passiflora* species. + indicates that substantial feeding took place, — that little or no feeding occurred. *Heliconius* data from Smiley (1978b). See legend Table 1 for further explanation.

Herbivore species	*Passiflora* species									
	A	D	G			P				
	PIT	VIT	AMB	QUAD	OER	LOB	COR	AUR	BI	COST
H. cydno	+	+	+	+	+	−	+	+	+	+
H. hecale		+	+		+	−		+	+	+
H. ismenius	+	+	+	+	+	−		+	+	+
H. melpomene		+	+		+	−		+	+	+
H. charitonia	+	+	−	−	+	+	+	+	+	+
H. erato	+	+	−	−	−	−	+	+	+	+
Alticini sp. 1		−	−		−	−		+	+	
Strabala sp. 1		+	+		+	−		+	+	
Strabala sp. 2		−	−		+				−	

difference, the two feeding guilds showed remarkable similarities. Both guilds possessed two relatively generalist species which use *Passiflora* in the two primary subgenera *Granadilla* and *Plectostemma*, and both possessed several additional species which were more specialized. Of these, one species was relatively abundant which specialized on *Granadilla*, while four species were found to specialize on *Plectostemma*. Both groups were also found to have 2-3 rare species whose host plant relations were not evident in the data.

Diversity comparisons (Table 2) also indicated remarkable similarity between the two groups. In the clearing habitat, *Passiflora*, *Heliconius*, and flea beetle diversities were quite comparable, as were diversities in secondary forest and edge habitats. In primary forest the flea beetle and *Passiflora* diversities were comparable, but the *Heliconius* diversity was greatly reduced. The diversity of *Heliconius* and flea beetles summed over all habitats was nearly identical, although somewhat less than the calculated *Passiflora* diversity. Overall, the two guilds were almost identical in diversity in each habitat, except for the *Heliconius* in primary forest where only two species were common.

Extensive data from *Heliconius* feeding trials (Smiley, 1978a), summarized in Table 3, indicate that *Granadilla* specialists can also consume *Plectostemma* successfully, but that the reverse is not true. This same result was observed in the flea beetle feeding trials. *Strabala* sp. 1, a *Granadilla* specialist, was observed to feed readily on *Plectostemma* as well as *Granadilla*, whereas Alticini sp. 1 and *Strabala* sp. 2, both *Plectostemma* specialists, were found to be unable to consume *Granadilla* host plants. Therefore it appears that both guilds are subject to similar constraints in the evolution of feeding tolerances.

Conclusions

The *Heliconius* were found to be somewhat more specialized in their host plant use than the flea beetles, which tended to use 2-3 species of host rather than the one host used by most *Heliconius*. Although this might be expected in the guild with the strongest evolutionary association to the host plants, it may also be an artifact of the much greater mobility of butterflies in seeking out host plants. A female butterfly such as *Heliconius melpomene* may pass over many edible host plants in searching for just the right species (Smiley, 1978b), but a flea beetle with its reduced powers of movement may be forced to occasionally consume any plant that is edible to it.

In spite of the observed difference in host specificity, the other characteristics of the two feeding guilds were remarkably similar. In diversity measures, in host plants used, in the response to host plant subgenera, the two guilds were nearly identical. The data indicate strongly that detailed characteristics of herbivore guilds may be determined by the host plant community, regardless of the degree of evolutionary association between insect and host plant. Therefore, long-term evolutionary associations such as those described by Ehrlich & Raven (1964) and Benson et al. (1976), do not necessarily have a major direct effect on the development of herbivore communities. However, an indirect effect, such as might be caused by coevolution acting to increase host plant diversity, has not been ruled out and must still be considered a strong possibility in this system.

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Secondary plant metabolites – round-table discussion

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Among the thousands of compounds synthesized by and stored within higher plants are a number of natural products that function in plant chemical defence. These compounds and others have come to be called allelochemics, although I much prefer the term: allelochemical, to indicate their role in organismic interaction of an interspecific nature.

As a beginning to our collective deliberations, the point was made that investigations of plant allelochemical interaction with insects are affected by several variables such as their concentration within the plant. Variability in the concentration of plant phenolics, for example, is very significant and appears to be responsive to changes in light intensity. In a given forest tree, the phenolic level of so called 'sun' leaves is greater than for comparable 'shade' leaves within the same plant; phenolic levels also differ in response to an altitudinal gradient. Similarly, in certain rain forests of Africa there is a correlation between high phenolic content and exposure to sunlight. Canopy trees have much higher levels of these natural products than do the understory shrubs. These findings may reflect light-mediated induction or activation of certain critical biosynthetic enzymes that support the production of these compounds. By contrast, the content of hydrogen cyanide, reflected in the storage of cyanogenic glycosides, markedly decreases with greater light intensity.

Variation in soil nitrogen appears to affect the level of secondary plant metabolites as well. The phenolic contents of plants growing on poor soils is relatively high. On the other hand, the level of compounds which are derived from amino acid precursors like cyanogenic glycosides, is increased by fertilization. These plant compounds, many alkaloids, non-protein amino acids, and certain other secondary compounds would be expected to be linked closely to the nitrogen availability of the plant.

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Variation in allelochemical content can be quantitatively significant and this must not be forgotten in allelochemical-insect investigations. No single factor accounts for the observed differences in natural product concentration; rather, this variation is a reflection of a multitude of concurrently interacting and interrelating factors.

Several members of the audience emphasized that it was important to be fully aware of the fine line that can divide and distinguish allomonic from kairomonic constituents. Simply stated, an allomone is an allelochemical that elicits a deleterious effect in the receiving organism. Kairomones, however, have beneficial effects in the receiving organism. This point can be addressed by examining certain pyrrolizidine alkaloids. Certain normally toxic compounds of this group are not only consumed by specialized insects but also sequestered for their own defensive purposes. This is an excellent example of a deleterious substance that assumes a kairomonic role in a particular insect. Recently, pyrrolizidine alkaloids were found to have striking morphological effects in *Creatonotos gangis* and *C. transiens*. These nitrogenous compounds regulate the growth of male scent organs and also serve as scent precursors. Males of these arctiid moths expand large, abdominal, hair-covered tubes (coremata) in the presence of a living female. Coremata weights can vary from 0.2 to 2.0 mg and the quantity of 7-hydroxy-6,7-dehydro-5H-pyrrolizine-1-carboxaldehyde (hydroxydanidal) found within these insects correlates well with coremata size. The biosynthesis of hydroxydanidal is related to the occurrence of pyrrolizidine alkaloids in the insect's foodstuffs. Thus, if larvae have adequate access to pyrrolizidine alkaloid-containing plants, full development of the coremata is assured since sufficient hydroxydanidal is synthesized from pyrrolizidine alkaloid precursors.

L-Canavanine is a deleterious non-protein amino acid of certain leguminous seeds. The bruchid beetle *Caryedes brasiliensis* is a highly adapted predator of the canavanine-laden seed of the leguminous vine *Dioclea megacarpa*. This insect has very active arginase and urease activity which converts much of the stored nitrogen of canavanine to ammonia via urea. Feeding studies utilizing ^{15}N-labeled urea reveal appreciable incorporation of this heavy isotope into a variety of insectan protein amino acids. Seed canavanine is an effective dietary nitrogen source for the developing larvae and thus an important kairomone for this particular insect.

In fact, use of the term allomone is not without some measure of intellectual difficulty. If a given natural product is successful in excluding a potential herbivore, it is hardly correct to call it an allomone; for, if the insect does not feed on the plant, a term like allomone is meaningless. On the other hand, if the natural product lacks the capacity to exclude the herbivore and the producing organism becomes a host plant, then it is equally inappropriate to call it an allomone.

The view was expressed that a parallel may exist between the above behavioural observations noted with hydroxydanidal and puddling behaviour in certain butterflies. Puddling behaviour refers to the observation of butterflies that land in mud puddles and then engage their probosci. If the soil is dry, they will exude a droplet which acts as a vehicle for nutrient uptake. Males acquire these nutrient resources and could pass them to the females via their spermatophore and thus enhance the potential reproductive success of the female. It is conceivable that there may have been selection for females being able to recognize males that have acquired such

nutrients. Pyrrolizidine alkaloids might act as 'sign stimuli' for males that have secured the necessary nutrients and therefore confer augmented reproductive success on the female.

Another panelist raised the matter of the lack of clear distinction between the following two points. On one hand, the presumed historical role of secondary substances as physiologically active selection agents in the evolution of host specificity in phytophagous insects and their actual role in the 'chemical information pattern-stimulus complex' to which the insect responds behaviourally by initiating feeding or oviposition. He continues: 'This distinction is of decisive importance since non-host plants are readily recognized, especially by specialist feeders, before any physiological effect can take place. In most cases without any attempt at feeding or biting. And by ovipositing females almost always after a simple contact.'

The chemical composition of the surface of the plant part, i.e. the 'halo' of compounds covering these parts may contain the message 'read' by the insect. The chemical nature of these chemicals is little known. Thus, it is not clear how many secondary metabolites, which are stored in the vacuoles or recycled in the plant's metabolism, but which are taken to function in plant chemical defence, actually participate in the insect recognition process that occurs at the plant surface. This panelist concluded by stating: 'It is very likely that each insect species perceives different chemical characteristics of the same plant. This perceived 'biochemical profile' probably involves not only different primary and secondary substances but also their various interactions at the chemoreceptor level. There is no way to predict whether certain secondary compounds or groups of compounds, that are regarded as typical for a given plant taxon, participate at all in the chemical information readable by a certain insect, i.e. whether they play any role in the plant's defence mechanism against that insect.'

It was noted that an insect may not cue in on a particular secondary metabolite but rather a compound associated with this metabolite. That is, an insect may learn to associate the occurrence of a particular secondary metabolite with something found on the surface of the plant and which is not itself noxious. Insects have relatively few receptors, perhaps because they do not need to tune into a variety of secondary metabolites but rather to a much more limited array of 'indicator compounds'. An audience member commented on his studies of a leafminer of soybean that had to contend with sclerenchyma tissue in the cortical area of resistant varieties. Certain colour-producing flavanoids delineate where tissue differentiation has occurred and thus where the sclerenchyma is to be found. Insect tuning into the appropriate colouration of the stem, would facilitate decisions as to where ovipositional activity should occur. It was also observed that many compounds that are detected by sensory systems and which have been shown to be effective deterrents, simply are not toxic. Similarly, many supposedly toxic compounds are not perceived by insects nor are they effective feeding deterrents. Once again, a caution flag was waved with regard to the need to guard against overly agressive generalizations in considering and characterizing allelochemical-insect interactions.

The final point raised from the audience dealt with the question of the existence of meaningful evidence for a coevolutionary interaction between plants and phytophagous insects. It is evident that plants have evolved many distinctive strategies

of chemical defence. The germane question is one of evidence that insects have co-evolved in direct response to these factors. Experimental evidence bearing on this question was offered by citing several examples. The neotropical leguminous tree *Hymenaea courbaril* is subject to bruchid beetle predation while its counterpart in Puerto Rico enjoys freedom from predation by this insect. The mainland population shows considerable physical evidence such as resin-impregnated seed pods of its adaptation to this seed predator. These physical features are lacking the Puerto Rican population. This argument is predicated upon the premise that if the selection pressure of this seed predator had not been exerted, the various chemical and physical defences would not have been acquired. Another example is that of glandless and glandular cotton. The glandular cotton with its allelochemical constituents is more productive agriculturally than is the glandless genotype. Finally, the susceptibility of glucosinolate-free cruciferous plants to herbivory relative to glucosinate-protected plants was cited. These examples and the classical study of David Jones with populations of *Lotus corniculatus* polymorphic for the genes controlling cyanogenic glycoside production provide a data base consistent with the belief that plants evolved protective allelochemicals in response to plant chemical defensive strategies. However, true coevolution in terms of the herbivore, remains illusive and lacking in substantive experimental support.

Plant resistance

Plant resistance to insects: a challenge to plant breeders and entomologists

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Abstract

The recent use of pesticides in selection fields threatens the maintenance of plant resistance to insects in future cultivars. It is demonstrated that current breeding populations are still variable for resistance to insects, but these differences should be better exploited. By slowing down the population development of insects, plant resistance is a potent component of integrated pest control. Several examples are given, including plant characters which promote the activity of parasites and predators. Developing resistant cultivars is most successful in close cooperation of plant breeders and entomologists. A general framework of a resistance breeding programme demonstrates that support of entomologists is most essential in developing test methods and in unraveling the mechanisms of resistance. This is illustrated on the resistance of cucumber to the twospotted spider mite, in which much attention is paid to the effect of the bitter compound cucurbitacin.

Key-words: plant resistance, pest control, parasites, predators, *Tetranychus urticae*, cucurbitacin, *Psila rosae*, *Trialeurodes vaporariorum*, carrot, tomato

Theoretically hundreds of thousands of plant species and hundreds of thousands of phytophagous insect (and other arthropod) species form a potential for millions of insect-host plant relationships. Perhaps there has been an era far back in evolution, when plants were almost unprotected and foraged by many, mainly polyphagous, insects (Feeny, 1975). These uses of food rather than host plants by undiscriminative herbivores can, however, hardly be considered as relationships. Owing to evolutionary processes most plant species are nowadays non-hosts for the majority of insect species. This specialization, which has led to more or less intimate relationships, benefits the survival of both plants and insects thanks to a partitioning of the available resources.

During evolution plants have been equipped with many physical and chemical defence mechanisms and resistance is the rule rather than the exception. Even a 'susceptible' plant is not necessarily an optimal host for the insect concerned. We found, for example, that removing the glandular hairs of a tomato cultivar known as very susceptible to the glasshouse whitefly *Trialeurodes vaporariorum*, increased the reproduction of this insect by 65%. This illustrates the potential for increased sus-

ceptibility due to careless manipulations of insect-plant relationships. It is the purpose of this paper to make people aware of these risks, and more important, to show the perspectives of a deliberate manipulation in the other direction, the breeding of cultivars resistant to insects and mites.

The cultivar as a variable factor of the agro-ecosytem

In a natural environment the realized growth rate of an insect population remains far below the potential growth rate. Complex biocenoses of host and non-host plants, phytophagous insects, parasites, predators and entomophagous microorganisms prevent serious pest developments, unless these stable ecosystems are disrupted.

Agriculture, especially modern agriculture, is an extreme form of ecosystem disruption. Plants are grown in monocultures under optimal conditions using fertilizers, irrigation, pesticides and modern cultivars. Without pesticides the realized growth rate will easily approach the potential one, resulting in dramatic crop losses. The contribution of cultivar differences to the prevention of insect outbreaks is often underestimated as the plant is considered as a static factor, neglecting its genetic diversity for many characters including its suitability for phytophagous insects.

Owing to varying selection forces this suitability has changed during evolution. Selection pressure in wild ecosystems towards resistance has only been moderate because pest populations are generally low in such balanced systems (de Ponti, 1977a, 1982a). This selection pressure increased markedly during the process of domestication in the early days of agriculture, when plants were more and more isolated from their protective environment and moved to virgin areas where pests could freely develop. In addition plant types were favoured which were potentially more susceptible by the absence of physical and chemical defence mechanisms derogatory to the consumptive quality. This urged the early farmers to collect those plants as seed parents for their next crop which were least attacked. This age-old selection for resistance continued into the first decennia of this century.

The above considerations suggest that centres of domestication are more valuable sources of resistance than centres of origin. Our experience in selecting carrots for resistance to the carrot fly *Psila rosae* demonstrates the contrast between natural and farmer's selection. In field screening 52 wild carrot accessions collected in the Netherlands and Israel their average level of attack hardly differed from that of the most resistant cultivars (47 vs. 49% carrots attacked in 1979), despite the woody roots of the wild carrots and the fleshy roots of the cultivated ones. The selection pressure of the carrot fly on the wild carrot is evidently rather low: as an annual plant it flowers and bears seed in one year even when the roots are heavily attacked. The biennial cultivated carrot, however, flowers in the second year and attacked carrots hardly overwinter. Therefore this crop exists only thanks to a permanent selection for resistance carried out by generations of alert farmers.

Since the introduction of synthetic pesticides this picture has changed for the worse. Chemical control of especially insects and other arthropods became common practice in selection fields, so that possible differences in resistance were masked. Although there is little written evidence (de Ponti, 1982a), this might have caused a

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gradual loss of resistance in modern cultivars. Anyhow alertness is called for, especially because it appears still possible to find reasonable sources of resistance in current breeding populations. In testing a number of wheat cultivars currently used in Britain for resistance to the cereal aphid *Sitobion avenae*, Lowe (1980) found significant differences in repeated glasshouse and field tests. Eenink & Dieleman (1977) and Bintcliffe (1981) had the same experience in testing commercially available lettuce and potato cultivars for resistance to the aphid *Myzus persicae.* These and many other differences could be better exploited by protecting selection fields from abundant pesticide use, so that at least natural and breeder's selection are restored (de Ponti, 1982a). Rapid results can be obtained by a deliberate selection for resistances as will be shown later.

From the above it is clear that in insect-plant relationships the genotype of the host is at least as variable as many other factors determining the 'success' of the relationship. The selection of resistant plants should be a challenge to both plant breeders and entomologists to the benefit of integrated pest control.

Host plant resistance as a basis of integrated pest control

Integrated control aims at keeping pest populations below damaging levels indicated by economic injury levels. Generally some insects can be tolerated without detrimental effect on yield and quality of the harvested product. Two categories of crops form an exception, where a zero-tolerance is requested: leafy vegetables and some ornamental crops such as roses and chrysanthemums. Because zero-populations are hard to achieve by integrated control, as system being increasingly preferred by many consumers and policy-makers, one might wonder whether in the future this zero-tolerance will remain as important as it is today.

Integrated control is most effective on pest populations which develop slowly and with limited fluctuations. On susceptible cultivars, pest populations can develop explosively which affects the stability and so the reliability of integrated control systems. Host plant resistance seems to be one of the best methods for keeping pest populations at controllable levels. Therefore it is recommended as an essential part of integrated control.

Host plant resistance reduces the reproduction rate in an insect population. The effect of this reduction on pest development over a number of generations and years has been calculated by van Emden (1966) and Knipling (1979) using theoretical models. Even low levels of resistance appear to be very effective by a cumulative effect. These models have been confirmed by many experimental data. From our own work I will show three examples based on − sometimes preliminary − glasshouse and field studies on the resistance of cucumber to the twospotted spider mite *Tetranychus urticae*, of tomato to the glasshouse whitefly *Trialeurodes vaporariorum* and of carrot to the carrot fly *Psila rosae*.

As demonstrated in Figure 1 the population or damage development on the most resistant lines is slower than those on the susceptible standard cultivars. This is a better proof of the benefits of resistance than any static measurement of a survival, reproduction or mortality factor as it shows the effect on the epidemic of the insect concerned. In combination with economic injury levels it is also possible to indicate

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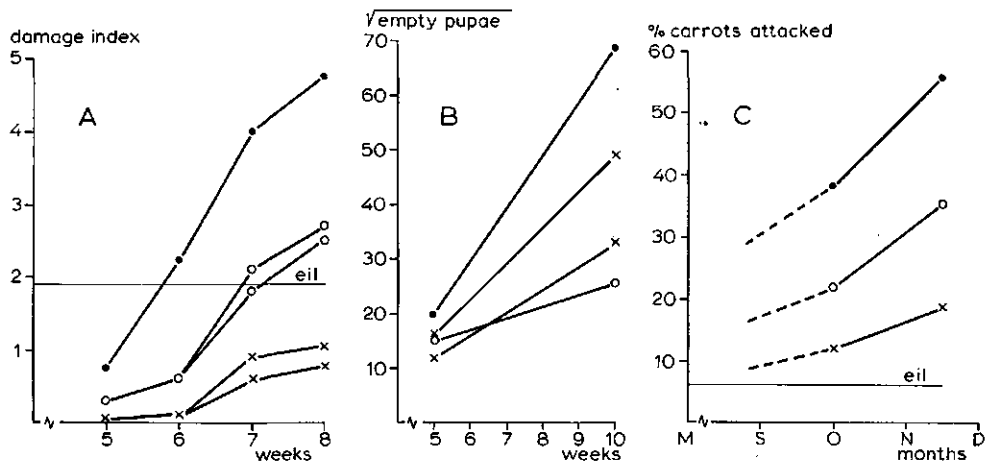
Fig. 1. Three examples of the population or damage development on susceptible standard cultivars (•), on partially resistant cultivars or related wild species (○), and on resistant selections (x). If known, the economic injury level (eil) is indicated. A: cucumber as attacked by the twospotted spider mite, B: tomato and the glasshouse whitefly, C: carrot and the carrot fly.

if and when additional control measures are necessary. The characteristic slow development of pest populations on resistant cultivars will markedly increase the efficacy and profitability of other integrated control measures (de Ponti, 1982b).

Although resistance is certainly the most obvious contribution, plant breeding can also promote integrated control in a different way. By changing specific morphological characters of a plant the activity of parasites and predators can be improved (de Ponti, 1982a). Hulspas-Jordaan & van Lenteren (1978), and de Ponti (1980a) reported that the parasitic wasp *Encarsia formosa* walks 3.5 times faster on a glabrous cucumber leaf than on a normal hairy leaf. This increased mobility is expected to enhance the parasitization of the glasshouse whitefly (*T. vaporariorum*) in such a way, that biological control of this insect on cucumber might become feasible. Recently we found that F_1 hybrids of a hairy and a glabrous parental line, which can easily be bred, have only half the number of hairs. The effect of such a reduction on the parasitization is subject for present research.

Breeding resistant cultivars by joint action of plant breeders and entomologists

This symposium is an ideal place to stimulate cooperation between plant breeders and entomologists for the development of insect-resistant cultivars. Such a team approach has proved to be very essential in achieving progress in this interdisciplinary area. The cooperation will only be successful if it is based on knowledge of or at least interest in the separate disciplines. This can cause difficulties at the start of a project because the two disciplines are rather isolated at many universities, although, for example at the Agricultural University of Wageningen, there is a grow-

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ing interest among students in following both courses, studying insect-plant relationships with a basic and an applied interest.

A general framework for the development of insect-resistant cultivars will indicate where entomology and plant breeding interact. The chronology of the six phases is obvious, but remains flexible.

Phase 1: Preparatory study of the insect-plant relationship in its entire biocenosis In this phase an inventory is made of the insect problems of a specific crop in relation to the natural control potential of their parasites, predators and entomophagous microorganisms. Primary and secondary pests are distinguished and the impact of a reduced population development by plant resistance on the future pest status estimated in comparison with other tactics of control. By population growth simulations it is possible to predict which level of resistance is required to achieve a significant shift in the development of the target insect. In a rather early phase of the project it can then be investigated whether such a level of resistance is achievable.

Crops with only one or a few pests are rather attractive for resistance breeding, whereas within a multitude of pests resistance to only one pest will hardly enable the introduction of a new protection strategy. This phase has a strong entomological emphasis, but the plant breeder should already be involved and search the possible sources of variation of the crop concerned. He should already be acquainted with the biological and behavioural features of the insect concerned.

Phase 2: Search for sources of resistance The available germplasm is evaluated by a system of consecutive tests of decreasing germplasm numbers and increasing sampling intensity and accuracy. Plant breeders have much experience with such tests, which should primarily be carried out under field conditions to avoid the selection of artifacts owing to irrealistic selection under artificial conditions. Entomologists will contribute in chosing the site and conditions of the field favourable for pest development. Preplanting or interplanting of susceptible host plants, mass releases of the target insect, selective control of parasites and predators, and other tactics can be applied to promote a certain and regular pest development throughout the field. The experimental design and scoring methods require careful consultations between all persons involved, whereby compromises are inevitable.

Usually local cultivars will be screened first, because a resistance found in such material can easily be incorporated in future cultivars (Buddenhagen & de Ponti, 1982). If still necessary foreign cultivars and related species are screened as additional reservoirs of resistance. International germplasm collections can be very useful in obtaining wide genetic diversity.

This phase will hopefully end in the detection of sources of resistance, one of the two basic prerequisites of a routine breedling programme. The other is the availability of an efficient and reliable test method.

Phase 3: The development of test methods based on a study of the insect-plant relationship This phase is the keystone of the whole project and will heavily rely on entomological support. Having available genotypes with different levels of resist-

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Table 1. Differences in population growth components of the twospotted spider mite on the most susceptible and the most resistant cucumber genotype.

Population growth component	Measure	Genotype		Proportional difference
		susc.	res.	
Adult survival	%/10 days	80	30	−62%
Adult longevity	days	24	16	−33%
Oviposition	eggs/ ♀/3 days	21	7	−67%
Juvenile mortality	%	3	30	+900%
Duration of development	days	9.5	10.5	+ 10%

ance to a particular pest, an analysis of these differences by lifetable studies will indicate what aspects of the relationship prevent the normal pest development on a resistant genotype. Studying the resistance of cucumber to the twospotted spider mite *Tetranychus urticae*, de Ponti (1977b, 1979, unpubl.) investigated most components of this relationship on susceptible and resistant genotypes. The extreme values are summarized in Table 1.

Based on statistical analyses of these data and technical considerations adult survival and oviposition were chosen as criteria for resistance in laboratory tests (de Ponti, 1977b). Later, selection was restricted to oviposition only. Similar procedures have been followed for resistance of tomato to the glasshouse whitefly *T. vaporariorum* (Berlinger & de Ponti, 1981) and for resistance of lettuce to the leaf aphids *Myzus persicae* and *Nasonovia ribis nigri* (Dieleman & Eenink, 1981; Eenink & Dieleman, 1981).

Although these studies provided valuable data and the choice of the selection criteria seems legitimate, the population dynamic consequences of the differences observed have not contributed to the decision about the most adequate selection criteria. Such consequences might throw new light on the relative value of these criteria. By simulation and field studies of *Sitobion avenae* on winter wheat Carter & Dixon (1981) demonstrated that a difference in one component of the insect-plant interaction gave a super-proportional response in the course of the population development, whereas a similar difference in another component gave a sub-proportional response. Evidently the former component is a better selection criterion for resistance than the latter. Therefore Carter & Dixon's plea for the use of sensitivity analyses in the evaluation of selection criteria deserves support. Using simulation expertise available in several institutions in the Netherlands, our host plant resistance projects will enter a new, dynamic and fascinating phase.

Based on a thus increased knowledge of the insect-plant relationship adequate field, glasshouse and laboratory tests can be designed. The correlation between these tests will also provide valuable information on their efficiency and reliability.

Phase 4: Breeding resistant cultivars Now that in the previous phases resistance resources and test methods have become available, the real breeding can start,

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Table 2. Population growth components of the twospotted spider mite on two nearly isogenic cucumber cultivars.

Cultivar	Cucurbi-tacin	Adult survival (%)	Oviposition (per ♀, 3 days)	Juvenile survival (%)
Improved Long Green	present	45	13.4	70.4
Mutant Improved Long Green	absent	37	13.2	70.1

which will heavily rely on plant breeders' expertise, because it does not essentially differ from any other breeding programme. Therefore I will confine myself to just mentioning some of the aspects of such a programme:
— increasing the resistance by intercrossing partially resistant resources
— transfer of resistance genes from related species to the cultivated one
— investigating genetic aspects of the resistance, such as the inheritance, heritability, gene actions, linkage with other characters
— combining the resistance with all other agronomic characters which make a modern cultivar

Phase 5: Investigating the backgrounds of the resistance This phase may enjoy great interest among many entomologists, but often suffers from insufficient involvement of plant breeders. Therefore the choice of the material is often inadequate. Analyses of just a resistant cultivar as compared with just a susceptible one will always show differences in the chemical or physical constitution, but give no information about the causality of these differences and the differences in resistance and susceptibility.

Based on a study of only two cucumber cultivars and crosses between them, Dacosta & Jones (1971a, 1971b) concluded that the bitter principle cucurbitacin is responsible for resistance to the twospotted spider mite *T. urticae* and susceptibility to the cucumber beetles *Acalymma vittatum* and *Diabrotica undecimpunctata howardi*. Amongst hundreds of cucumber cultivars screened for resistance of the twospotted spider mite we found, however, many susceptible and bitter cultivars, and we questioned Dacosta and Jones' hypothesis (de Ponti, 1978). Having available nearly isogenic lines, which differed only in the presence or absence of cucurbitacin we possessed unique material to test the effect of cucurbitacin. Both in laboratory tests (Table 2) and glasshouse tests (Figure 2A) no effect of cucurbitacin could be detected and any causal relation between resistance and bitterness was denied (de Ponti, 1980b). Nevertheless a disproportional surplus of resistant and bitter plants in an F_2 after crossing resistant and bitter with susceptible and non-bitter cultivars and lines was found (de Ponti & Garretsen, 1980). Therefore we hypothesized a genetic relation in terms of linkage of genes for bitterness and resistance. Breakage of such a linkage would enable the selection of non-bitter and resistant plants and lines. Such lines were recently selected (Figure 2B).

Analogously Lower (1972), and Quisumbing & Lower (1978) found cucumber cultivars which were non-bitter and susceptible to the earlier mentioned cucumber
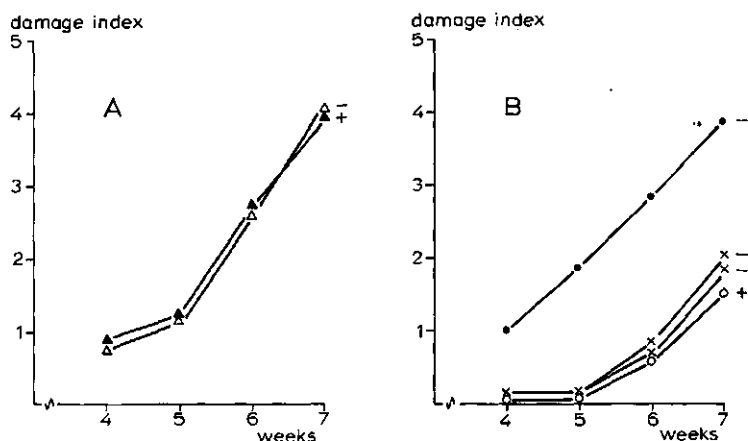
Fig. 2. Increase in damage index after inoculation with the twospotted spider mite of (A) a pair of near isogenic cucumber cultivars which differ only in the presence (+) or absence (−) of the bitter principle cucurbitacin, and (B) of a non-bitter and susceptible cucumber cultivar (•), a resistant and bitter breeding line (o) and two non-bitter and resistant breeding lines (x) derived from a cross between these two parents.

beetles. This example illustrates how careful one must be in ascribing resistance to just a single compound, especially if the plant material investigated was limited.

The benefits of a close cooperation between plant breeders and entomologists is demonstrated in our work on resistance in carrot to the carrot fly *Psila rosae*. By field testing in an area where the pest is generally very active we have selected cultivars and breeding lines which are less attacked than many of the cultivars currently grown in Europe (de Ponti & Freriks, 1980). Cultivar differences have been confirmed by multilocational tests in five European countries in two consecutive years (Ellis & Hardman, 1981). Because of the relatively small amounts of seed available after selection and the large numbers to be tested, the primary screenings do not allow large plots. Therefore in our programme the material was tested in eight replicates of one 1.5-metre row and classified at harvest as attacked or non-attacked. We suppose that such an experimental design does not allow any discrimination by the flies of volatile differences, so that the differences in attack (Table 3) are probably mainly due to differences in antibiosis to the larvae.

This would mean that a very potent factor, which influences the attractiveness of the carrot for oviposition has not yet been selected for. Based on results of Städler (1972), Guerin & Visser (1980), Guerin & Städler (p.95), and Städler & Buser (p.403), Visser and Schaefer analyzed some of our most resistant selections for differences in the oviposition stimulant trans-methyliso-eugenol. Some susceptible cultivars and populations were added. Table 3 shows some of the data, which indicate that there is no relation at all between the percentage of carrots attacked and the amount of trans-methyliso-eugenol. Selection for low levels of this and other oviposition stimulants, of volatile and non-volatile nature, will add a new dimension to this project. Thus we attempt to disrupt the insect-plant relationship at several

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Table 3. Comparison of the proportion of carrot plants unattacked by the carrot fly in a field test in 1980 and the amount of trans-methyliso-eugenol in their leaves (data from Visser, de Ponti & Schaefer, unpubl.).

Material	Carrots unattacked (%)	Trans-methyliso-eugenol content (mg/kg)
Population Nantes 79609	45	13.40
Population Nantes 79617	63	14.15
I_2 Vertou	72	0.30
Population Nantes 79611	73	16.25
I_2 St. Valery	74	7.75
Population Signal 79660	85	2.93
I_2 Vertou	89	3.20

places, expecting an accumulative effect on the resistance level.

This phase will have a feedback on Phase 3 in improving existing test methods or adding new ones. It will also contribute to the next phase by providing information on possible effects of the resistance mechanism on the food quality for human or animal consumption.

Phase 6: Evaluation of the newly bred resistant cultivars Now that resistant cultivars have been bred, they have to be incorporated in integrated pest control systems. The compatibility with other integrated control tactics should be investigated. Especially possible negative effects on biological control by parasites and predators as indicated by Barbosa (p.63) deserve attention. If insecticides are still necessary, then they can be used at a lower rate and lower dosages, which should be determined in order to inform the farmers about the use of these new cultivars.

The introduction of new resistant cultivars is one of the easiest tactics of integrated control, because it needs hardly any guidance of extension services. Farmers are well aware of the benefits of resistance by their experience with disease-resistant cultivars. They are generally willing to adopt such new cultivars provided the standards for all other agronomic characters are also met. This last point is often forgotten by those who are not familiar enough with the agricultural practice.

Conclusion

Returning to the title of this paper I hope that more entomologists will accept the challenge to cooperate with plant breeders to develop insect- and mite-resistant cultivars. This symposium has shown again that there is a wealth of knowledge of insect-plant relationships, which could better serve the solution of pest problems in agriculture. Needless to say that basic research in insect-plant relationships will continue to form a reservoir of knowledge on which future breakthroughs are dependent.

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Resistance of Lactuca accessions to leaf aphids: components of resistance and exploitation of wild Lactuca species as sources of resistance

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Abstract

Lettuce growing is greatly hampered by the leaf aphids *Nasonovia ribis nigri* and *Myzus persicae*. Several accessions of the wild species *Lactuca virosa* appeared to be completely resistant to *N. ribis nigri*. Through a series of interspecific crosses resistance was transferred to the cultivated lettuce. Genetic research of the inheritance of resistance indicated that at least in a number of the resistant accessions of the wild species, resistance is governed by one dominant gene always located at the same locus. Partial resistance to *M. persicae* was found in several *Lactuca sativa* cultivars. Through genetic recombination the resistance level could be enhanced. Resistance is governed by additive genes. To distinguish susceptible and partially resistant plants various components of resistance were investigated. In the aphid-plant relationship resistance was measured by acceptance, larval and adult mortality, larval period, and larval production. Significant differences between plant genotypes were found for all these components. Investigations of the influence of temperature on the level of resistance did not reveal any interaction between temperature, plant genotype and aphid biotype.

Key-words: plant resistance, *Lactuca* species, *Nasonovia ribis nigri*, *Myzus persicae*, lettuce, reproduction, biotypes

Lettuce growing is greatly hampered by the leaf aphids *Nasonovia ribis nigri* (Mosley) and *Myzus persicae* (Sulz). Research on resistance is aimed at overcoming the above problems (Dieleman & Eenink, 1980).
The research programme is composed of the following parts:
— development of test methods
— screening a large collection of *Lactuca* accessions for genetic variation in resistance
— research on components of resistance, on their inheritance and on plant genotype - aphid genotype - environment interactions
— increase of the resistance level by genetic recombination
— research on chemical compounds which are responsible for resistance.

Table 1. Classified distribution of plants according to number of sur-
viving *Nasonovia ribis nigri* aphids, assessed 7 days after inoculation
of each plant with 10 first instar larvae (PIVT & cd are gene bank
code numbers). Results are pooled for three biotypes as no plant gen-
otype × aphid biotype interaction occurred.

Genotype	Origin	Number of plants										
Surviving aphids →		0	1	2	3	4	5	6	7	8	9	10
L. virosa												
PIVT 273	USA	1	1		2	1	3	1	1	1		
PIVT 275	Neth.	9	2									
PIVT 278	Neth.	11	3									
PIVT 280	Neth.	15										
PIVT 694	Switzerl.	2	2	1								
PIVT 701	W. Germany	13	1									
PIVT 714	Czechosl.	13	2									
PIVT 723	France	1										
cd 741004	unknown	8	6	1								
L. alpina												
PIVT 726	Sweden	7	6	1								
L. serriola	unknown	0	2	0	0	3	2	4	3	4	1	1

Leaf aphids

Nasonovia ribis nigri   The IVT *Lactuca* gene bank was screened for the presence of
resistant lettuce genotypes. Various screening methods were used (Eenink & Diele-
man, 1977; Dieleman & Eenink, 1981; Eenink & Dieleman, 1981). Accessions of the
wild species *L. virosa* appeared to be completely resistant to *N. ribis nigri*, except for
PIVT 273 which showed to be rather susceptible (Table 1). Through a number of
interspecific crosses resistance was transferred from *L. virosa* to the cultivated let-
tuce (Eenink et al., 1982b).

A series of diallel crosses was made within the *L. virosa* complex to investigate the
inheritance of resistance, multiple allelism, and the location of resistance genes.
Also crosses were made between resistant and susceptible lines of *L. sativa*. Ana-
lyses of some of the crossing populations revealed that at least some of the domi-
nant R-genes in *L. virosa* were present at the same locus while multiple allelism also
occurred (Table 2). Resistance transferred from *L. virosa* accessions to *L. sativa* was
also governed by one, dominant gene (Eenink et al., 1982c).

Myzus persicae   Partial resistance to *Myzus persicae* was found in several, mainly
old, *L. sativa* cultivars (Eenink & Dieleman, 1977) such as cvs. Plenos (PIVT 339),
Liba (PIVT 227) and *L. sativa* crisp (PIVT 619) shown in Table 3. The resistance of
these genotypes resulted in a significant reduction of about 50% of the intrinsic rate
of increase (r) of the aphid population compared to this rate on a susceptible gen-

Table 2. Classified distribution of parent plants and of F_2 populations according to infestation by *Nasonovia ribis nigri* aphids. + : susceptible; —: resistant; ± : intermediate.

Population	Number of plants							Total
Aphids per plant 15 days after inoculation →	0	1-2	3-6	7-14	15-30	31-60	61-120	
L. virosa								
PIVT 273 (±)			1	2	3			6
PIVT 731 —	3		1					4
F_2 (273×731)	38	30	33	11	20	4		136
PIVT 278 (—)	4							4
PIVT 714 (—)	4							4
F_2 (278×714)	139	7	2					148
L. sativa								
cv. Suzan (+)							9	9
F_2 (*L. sativa* × L_4 line)		130		10			54	194

otype for the most virulent biotype (WM1) on lettuce (Dieleman & Eenink, 1981).

An accurate determination of the actual population size on the basis of r is, however, hampered by various factors such as aphid density, plant age and temperature. For instance, young plants are more resistant than old plants (Eenink & Dieleman, 1980) and with high aphid density, larval production is smaller than with low aphid density.

Table 3. Classified distribution of plants of *Lactuca sativa* genotypes resistant or susceptible to *Myzus persicae*. Aphid infestation per plant was assessed on a scale from 0 (no aphids) — 9 (many aphids).

Species or cultivar	Gene bank code PIVT	Origin	Number of plants										Mean
Scale for aphid infestation →			0	1	2	3	4	5	6	7	8	9	
L. sativa	618	E. Germany	1	4	15	9	3	1					2.3
L. sativa	619	China		4	14	10		1					2.4
Plenos	339	Netherlands		4	17	12	3						2.4
PI 177418	370	USA/Turkey		4	8	14	8		3	1			3.2
PI 169497	364	USA/Turkey		9	11	11		1	1	2			2.6
Liba	227	Netherlands		1	4	8	5	1	1	1	2		3.6
Romana verde	508	Italy		1	4	10	10	4	1				3.6
Kakichisha	197	Japan				3	7	8	5	7	4	2	5.5
PI 250020	407	USA/Egypt				2	4	8	6	6			5.5
Taiwan native	313	China				4	4	7	11	8	3		5.6
Verde ricciol.	575	Italy						9	5	3	11	4	6.8

Table 4. Classified distribution of parent plants and of F_4 lines according to infestation by *Myzus persicae*. Aphid infestation per plant was assessed on a scale from 0 (no aphids) — 9 (many aphids).

Genotype	Number of plants										Mean
Scale for aphid infestation →	0	1	2	3	4	5	6	7	8	9	
L. sativa											
PIVT 619		4	14	10	1						2.4
PIVT 370		4	8	14	8		3	1			3.2
F_4 line (619 × 370)	3	15	9	5							1.6
PIVT 227		1	4	8	5	1	1	1	2		3.6
PIVT 508		1	4	10	10	4	1				3.6
F_4 line (227 × 508)		15	17	5	2						1.9

Diallel crosses were made between partially resistant plant genotypes and between partially resistant and susceptible plant genotypes to investigate the inheritance of resistance and to increase the resistance level. Crossing populations were analysed by using macrotests where aphid populations per plant were assessed and estimated on a 0-9 scale and by microtests where leaf cages were used with only a few aphids per cage. Characters like larval and adult mortality, aphid biomass and larval production functioned as criteria of resistance (Eenink & Dieleman, 1977).

Analyses revealed that resistance was governed by additive genes (g.c.a. effects) while almost no non-additive gene action (s.c.a. effects) was found. This implies that such additive genes can be accumulated in breeding lines provided they are different in the various partially resistant plant genotypes. Intercrossing such genotypes showed that in certain combinations no accumulation occurred because additive genes apparently were present at the same loci. With other combinations, however, intercrossing did result in an accumulation which gave a higher resistance level. This is shown in Table 4 for an I_4 breeding line from the cross *L. sativa* crisp (PIVT 619) × *L. sativa* (PIVT 370) and for an I_4 line from the cross Liba (PIVT 227) × Romana verde (PIVT 508).

Components of resistance

To distinguish susceptible and partially resistant plants, to increase the resistance level and to incorporate as many barriers as possible against the parasite, components of resistance were investigated such as: non-acceptance, mortality of larvae, mortality of adults, larval period, larval production, and frequency of winged aphids. Accumulation of several components could increase the level and thus the durability of the resistance if the components were based on different mechanisms and came from different genetic backgrounds (Eenink et al., 1982a).

Table 5 shows the differences between a completely resistant accession of *L. virosa* and a susceptible accession of *L. sativa* for acceptance by *N. ribis nigri*. Differences between plant genotypes for the acceptance component became significant particularly 10 hours or more after inoculation. This has been observed in both choice and no-choice conditions.

Table 5. Comparison of acceptance by *Nasonovia ribis nigri* between two *Lactuca* species. Percentage of winged aphids per plant at various hours after inoculation.

Genotype	Aphids per plant (%)							
Hours after inoculation →	1	4	6	8	10	12	14	22
L. sativa (susceptible)	12	18	32	39	65	53	54	80
L. virosa (resistant)	11	35	38	55	28	35	35	20

Table 6 shows the combined effect of acceptance or non-acceptance and adult mortality in the first 8 days after inoculation of resistant or susceptible plants with 10 winged adults of *N. ribis nigri*. On the resistant plant genotype no adults were found after 4 days while on the susceptible genotype, more than 50% of the adults were still present even after 7 days. It can also be seen that large differences occurred between resistant and susceptible genotypes in the number of larvae produced by these winged aphids present between 0 and 8 days after inoculation. On the resistant wild *L. virosa* all larvae had died after 7 days while on the susceptible genotype about 100 larvae were present on average. On the resistant *L. sativa* breeding line a limited number of larvae occurred eight days after inoculation. A few days later they had all died. Nevertheless, the resistant *L. sativa* line seems to be somewhat less resistant than the resistant *L. virosa* progenitor, implying that during the transfer of the major R-gene for resistance from the wild species to *L. sativa*, minor genes for resistance were lost.

The components of resistance assessed on a partially resistant and a susceptible plant genotype after inoculation with two biotypes of *Myzus persicae* (WM1, WM2) are shown in Table 7.

Only main effects (plant genotype and aphid genotype effects) and no interaction effects (plant genotype × aphid genotype effects) occurred which means cv. Plenos was always more resistant than cv. Taiwan and biotype WM1 was always more vir-

Table 6. Resistance of three lettuce genotypes to *Nasonovia ribis nigri*. Mean number of adults and larvae per plant between 0 and 8 days after inoculation with 10 winged aphids per plant per genotype. Means are based on 4 plants. +: susceptible; −: resistant.

Aphids	*L. virosa* (−)					*L. sativa* (−)					*L. sativa* (+)			
Days after → inoculation	0	1	4	7	8	0	1	4	7	8	0	1	2	7
Adults	10	7.3	1.5	0	0	10	6.5	1.5	0	0	10	7.3	6.8	5.5
Larvae	0	11.3	3.5	1.3	0	0	10.3	12.8	14.8	9.3	0	17.3	30	98

Table 7. Components of resistance of two lettuce genotypes measured after inoculation with two biotypes (WM1, WM2) of *Myzus persicae*.

Plant genotype	Insect weight (μg) after 7 days		Larval mortality (%)		Larval period (days)		Larval production in 10 days	
	WM1	WM2	WM1	WM2	WM1	WM2	WM1	WM2
Taiwan native (susceptible)	637	268	4	48	7.9	8.7	37.7	18.7
Plenos (partially resistant)	326	110	22	96	8.4	11.5	25.0	11.1

ulent on these two lettuce genotypes than WM2. Differences between plant genotypes were largest and most consistent for components like insect weight, larval period and larval production. On the susceptible genotype aphids were larger and heavier, larval mortality was less, larval period was shorter and larval production and survival rate of adults was higher than on the resistant genotype. This situation applies to both biotypes. From field screening experiments we obtained a third biotype (WM3), which was intermediate to WM1 and WM2 in its behaviour on susceptible and partially resistant lettuce genotypes.

Research on lettuce genotype - aphid biotype - temperature interactions for components of resistance has not revealed any such interactions so far. Temperature influenced components like larval period and larval production, and as they reacted differently for low (20°C) and high temperature (25°C) it may be concluded that various components for resistance are based on different mechanisms and thus different genes. Such a diverse and perhaps complex genetic composition of the resistance will increase its durability. Further investigations on the inheritance of resistance components are planned. Investigations have also been started to study backgrounds of resistance by analysing constituents of the phloem sap.

Conclusions

Within the genus *Lactuca* considerable genetic variation for resistance to leaf aphids was found. Resistance was found to be governed by single dominant major genes and/or various minor genes. Through genetic recombination the resistance level to *M. persicae* could be enhanced. A plant genotype - aphid genotype - environment interaction could not be detected. Because no resistance-breaking biotype of *N. ribis nigri* was found, the resistance is now being introduced into lettuce cultivars.

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Soybean resistance to beanflies

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Abstract

Multiple regression analysis has shown that the interaction of various plant parameters affect the level of beanfly infestation of soybeans. During early growth stages, beanfly infestation was affected especially by trichome density on the abaxial leaf surface, leaf area, leaf moisture and stem diameter. Resistance thus involves higher trichome density, smaller leaf area, higher leaf moisture and a narrower, more compact stem. When in the three-node stage or older, both a higher dry weight of the second trifoliate leaf and a higher moisture content in the internode between the second and third nodes also are correlated with soybean resistance. The plant parameters identified in this study have provided the necessary bases for a highly effective predictive model of beanfly resistance.

Key-words: Melanagromyza sojae, Ophiomyia species, stem diameter, soybean, trichomes, plant resistance

Agromyzid beanflies are major pests of legume crops in many tropical and sub-tropical areas of the world. The beanflies, *Melanagromyza sojae* (Zehntner), *Ophiomyia centrosematis* (de Maijere) and *O. phaseoli* (Tryon), infest 90-100% of the unprotected commercial soybean seedlings in such areas as southern Taiwan (Rose et al., 1978). Economical damage results from mining the plant stem, as well as the leaves, with resultant plant collapse. Field screening of 7 000 accessions identified four wild soybean (*Glycine soja*) Sieb. and Zucc. varieties as highly resistant to the above three beanfly species, and two accessions of *G. max* (L.) Merr. as susceptible to highly susceptible (Chiang & Talekar, 1980). Parameters of plant resistance versus susceptibility to insects range from morphological-anatomical to physiological-biochemical (Norris & Kogan, 1980). Characteristics of soybeans which determine resistance to beanflies are being identified (Chiang & Norris, 1982), and further recent progress is presented in this paper.

Materials and methods

Data on beanfly infestation and plant damage under field conditions were obtained in Taiwan (Chiang & Talekar, 1980). Selected plant parameters were com-

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Table 1. Comparative values for plant parameters correlated with soybean resistance to beanflies.

Parameter	Susceptible		Resistant			··
	G6	G1935	G3089	G3091	G3104	G3122
Trichome density [1]						
(number/cm^2)	107a	187a	318b	363b	381b	605c
Leaf area (cm^2)	24.3a	17.1a	4.7b	3.8b	3.5b	3.4b
Stem diameter (mm)	5.2a	2.7b	1.3c	1.4c	1.4c	1.5c
Leaf moisture (g/kg)	831a	832a	881b	866b	878b	866b
Leaf dry weight[2]						
(mg/cm^2)	2.6a	2.6a	1.0b	1.1b	1.2b	1.3b
Stem moisture[3]						
(g/kg)	908a	905a	752b	748b	798b	784b

1. Trichomes on abaxial surface of fully expanded leaves.
2. Measurements were on the second trifoliate leaves of V_3 plants.
3. Measurements were on the internode between the second and third nodes of V_3 plants.
Means in each column which are followed by the same letter are not significantly different at $P = 0.05$ (Duncan's multiple range test).

pared among the four resistant accessions, Nos. G3089, G3091, G3104 and G3122; and the two susceptible varieties, Nos. G1935 and G6, when grown under highly controlled photoperiod, temperature, relative humidity and nutrition in a biotron (Chiang & Norris, 1982). Thirty plants per accession were used as experimental material. Because critical beanfly infestation occurs in the seedling soybean, plant parameters were measured at three stages of plant growth, i.e., when the first (unifoliate) leaf, and first and second trifoliate leaves were fully expanded on the plant when it was in the one node (V_1), two node (V_2) and three node (V_3) stages, respectively (Lee, 1976). Investigated parameters were leaf thickness, leaf area, leaf moisture and dry weight, trichome density on the abaxial leaf surface, stem diameter and stem moisture (Chiang & Norris, 1982).

Results and discussion

The morphological parameters of trichome density on the abaxial leaf surface, leaf area and stem diameter; and the physiological characteristic of leaf moisture were all correlated with resistance during the V_1, V_2, and V_3 stages of plant growth (Table 1). The additional physiological parameters of leaf dry weight and stem moisture were correlated with resistance during the V_3 stage of plant growth (Table 1).

The linear correlation and linear regression between beanfly infestations and each of the above identified significant parameters of soybean resistance have been developed by Chiang & Norris (1982). Such regressions on trichome density on the abaxial leaf surface and on stem diameter are presented in Figures 1 and 2, respec-

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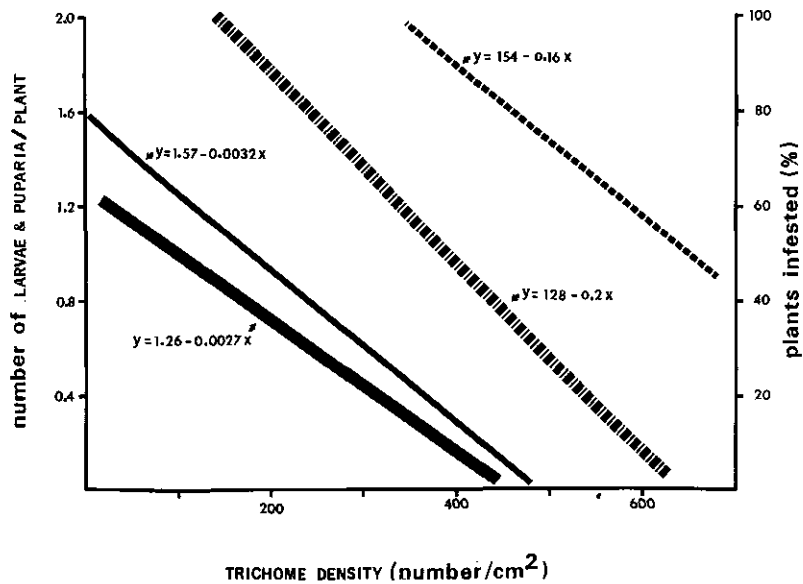
Fig. 1. Trichome density on the abaxial surface of the soybean leaf versus beanfly infestation; infestation at 3 weeks after seeding (thicker line), at 6 weeks after seeding (thinner line), number of larvae and puparia per plant (solid line) or percentage of beanfly-infested plants (dashed line).
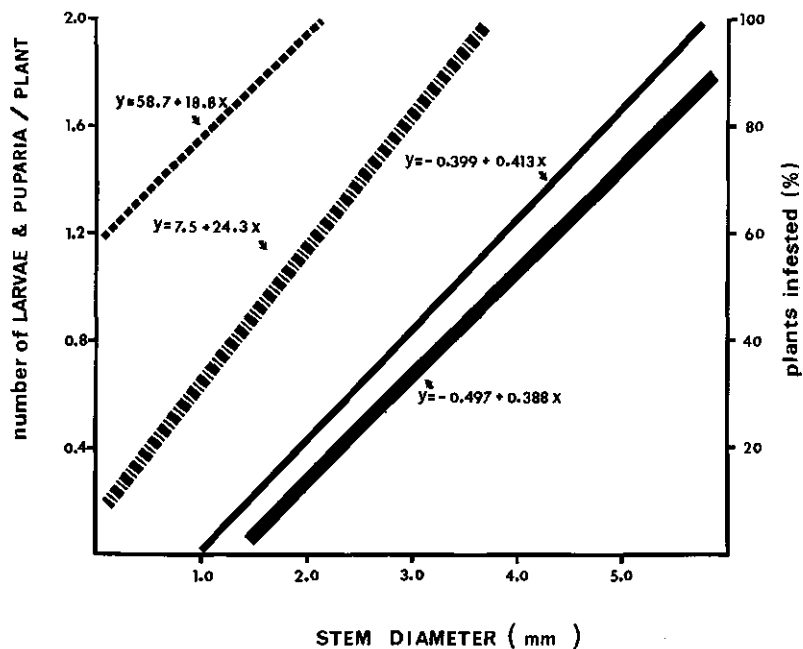
Fig. 2. Stem diameter versus beanfly infestation (see key to lines, Figure 1).

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tively. The observed regression of the percentage of beanfly-infested plants at 3 weeks on trichome density (Figure 1) indicates that a density of about 600/cm² or more on fully expanded leaves nearly completely deters oviposition. As the density falls progressively below this level, the contribution by trichomes to plant defences against beanfly decreases rather linearly.

The regression of beanfly infestation on stem diameter (Figure 2) is biologically interesting and significant because larvae of all three species of the insects mine down the stem through species-specific pathways (Chiang & Norris, 1982). The more compact stem of resistant varieties, with a smaller diameter (i.e., 1.3-1.5 mm, Table 1), apparently especially hinders the beanflies' activities in their last (i.e., third) larval instar and during pupation.

The correlation of beanfly infestation with leaf size seemingly agrees with the observation that adults prefer to lay eggs near veins, and a larger leaf presents more area adjacent to veins for oviposition (Chiang & Norris, 1982). The observed preference of beanflies for soybean leaves with a lower percentage of moisture is the opposite of the situation previously reported for mungbeans by Lin (1979). The demonstrated positive correlation (Table 1) between leaf dry weight/cm² in the second trifoliate leaves of plants in the V_3 stage and beanfly infestations is compatible with such leaves, which are preferred for oviposition at this plant stage, contributing to the better nutrition of newly infesting larvae. A relatively high moisture content in the internode between the second and third nodes, which is mined by young larvae as they move into the stem from the preferred second trifoliate leaf of V_3 stage plants, favours beanfly infestations (Table 1). This characteristic is probably associated with the fact that this internode in susceptible varieties in the V_3 stage is relatively undifferentiated in terms of secondary growth when compared to the resistant accessions (unpublished results). This observed difference in this internode between resistant and susceptible varieties in the V_3 stage is compatible with the concept of resistance being dynamically involved with the rate and extent of secondary growth and cell differentiation.

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Summaries of poster presentations

Food selection behaviour in Locusta migratoria

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Locusta migratoria is oligophagous, accepting most grasses and rejecting most dicotyledons. The sensilla chiefly involved in food testing are the contrast chemoreceptors on the palp tips and within the buccal cavity. The latter contact food when the insect bites. Palpation generally precedes biting and rejection may follow either of these.

In our experiments, locusts were caged individually and their reactions to potential food plants observed. Wheat was nearly always accepted but the unpalatable dicotyledons *Senecio vulgaris, Senecio jacobea* and *Brassica oleracea* were always rejected. In previous work *Bellis perennis* was rejected initially after palpation but, as hunger increased the further test of biting was made before rejection occurred. In the present work the reverse was the case; most insects palpated and bit before rejecting initially but on later contacts rejection mostly occurred after palpation only, suggesting that the insect learns to correlate the sensation at palpation with that at biting and later the rejection response is triggered by palpation alone. This was tested by observing the first eight contacts with unpalatable plants, using *S. vulgaris* as the plant all the time or, alternatively substituting *S. vulgaris* by either *S. jacobea* or *B. oleracea* after the first four contacts. When only *S. vulgaris* was used there was a progressive change from rejection at biting to rejection at palpation, according to the learning hypothesis proposed, but when either of the other two plants was introduced the pattern reverted to predominantly rejection at biting — the learning sequence was broken and started afresh. The break was more profound when a different plant genus was involved.

If learning and short-term memory play a significant part in food selection, this could have important implications for our understanding of the behaviour involved in the sequential sampling of potential food materials and in the assessment of its palatability. Many studies are based on the concept that on every test encounter the insect must make the decision to eat or not, on the basis of information obtained during that encounter only, regardless of the experience of recent tests and in igno-

rance of the presence and relative palatability of any adjacent potential food. The present work suggests that this assumption may not be warranted and experiments are in hand to examine the time sequence of this learning and its effect on food selection behaviour.

Olfaction at the onset of host plant selection

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As to study the role of plant odours in host selection by phytophagous insects three lines in research were undertaken: (a) chemical analyses of plant odours (in cooperation with the Central Institute for Nutrition and Food Research TNO), (b) electrophysiological recordings of the responses in insect olfactory receptor cells to plant volatiles and (c) behavioural observations of the insect's orientation towards plant odours. For a variety of phytophagous insects the leaf odour complex consisting of saturated and unsaturated C6 alcohols, aldehydes and the acetate ester, might play a substantial role in olfactory orientation. In phytophagous insect species the olfactory receptors are sensitively tuned to the reception of these volatiles, which are common constituents of leaf odour blends. Each of the insect species shows certain traits in the character of their sensitivity spectra for the green odour components (Visser, 1982).

As to continue the research on olfactory orientation a locomotion-compensator has been constructed. This instrument has been originally designed by Kramer & Heinecke. The locomotion-compensator operates as follows: A tiny piece of reflective material is affixed on the back of an insect. The insect is placed on top of the sphere and viewed by a camera. The camera projects a beam of visible or infra-red light onto the insect, which is reflected by means of the 'mirror' on the insect's back, thereby the position of the animal is detected. As soon as the insect starts walking its positional change is seen by the camera, which initiates that two motors rotate the sphere in the opposite direction with the same speed as the insect is walking -- the insect stays on top of the sphere while walking --. Pulse generators which make contact with the sphere, quantify the insect's movements, and in combination with a computer, walking speed and direction are calculated.

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Preference of Pieris brassicae for an unusual cruciferous plant species

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Key-words: Pieris brassicae, Alliaria officinalis, food preferences

Lepidopterologists rarely, if ever, found *Pieris brassicae* L. (Lep. Pieridae) on *Alliaria officinales* L. (Cruciferae), in nature in Hungary. Literature data (Gergely, 1947; Bergmann, 1952; Terofal, 1965) are also ambiguous on the matter. *Alliaria* is an early growing, biennial, onion-smelling weed at forest-edges and on N-rich ruderal places, featuring the characters of its family (Feeny, 1976). It is abundant in areas where *P. brassicae* occurs but restricted mostly to shady habitats. The insect sporadically occurs on *Alliaria* and prefers sunny habitats.

A short study was undertaken to clarify some aspects why *Alliaria* is not within the factual host range of this insect species. Rearing experiments and larval or adult preference studies have equally shown that *Alliaria* supports larval growth better and there is an overall preference for it as compared to savoy cabbage, irrespective of larval rearing history prior to tests.

In spite of this it seems probable that *P. brassicae* never, or very rarely exploits this potential host plant, because:
— Albeit there is no temporal isolation between the two species, differences in habitat preference reflect on substantial behavioural discord (Singer, 1971), and therefore result in a spatial separation;
— Patchy distribution of second year (stem) plants cannot provide adequate support for gregareously feeding young instars;
— Stem plants die away by the end of June, in Hungary, whereas alternative cruciferous plants are numerous by that time;
— No evidence is available for any relationships in the past for the two partners, and other herbivorous insects have also been found on *Alliaria*. Therefore, this study does not seem to support the theory of 'escape in space and time' (Feeny, 1976).

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Chemical isolation of feeding stimulants and deterrents from tomato for the tobacco hornworm

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Larvae of the tobacco hornworm, *Manduca sexta*, feed mainly on solanaceous plants. Our current research tries to determine which phytochemicals control feeding in this insect. A hexane extract of tomato leaves was screened for the presence of
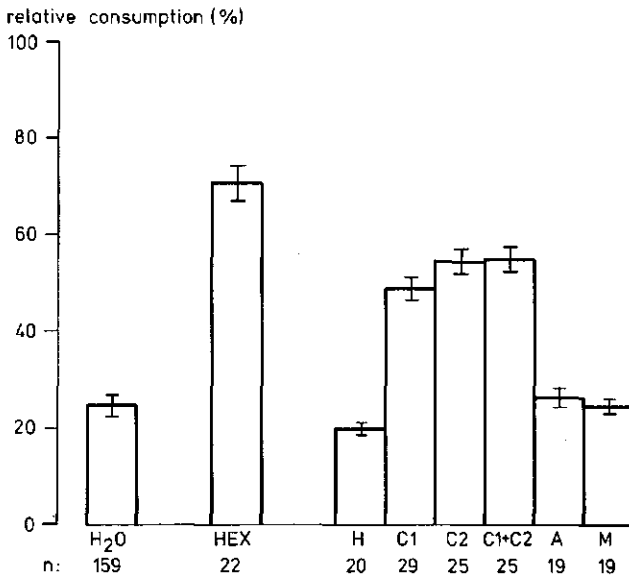
Fig. 1. Relative consumption of *Manduca sexta* larvae in a two-choice preference test. Individual larvae were offered the choice between a control diet (H_2O) and diets containing a hexane extract of tomato leaves (HEX) or fractions of this extract. H: hexane; C: chloroform; A: acetone; M: methanol fraction. *n*: number of larvae.

feeding stimulants and deterrents by a two-choice preference test. Tomato-reared fifth instar larvae showed a maximal preference for natural concentrations (IX), while threshold response was found near 10^{-3} X. Jerusalem cherry-reared larvae showed a completely different response to this extract. Preferences were weaker than those of tomato reared larvae and the dose-response curve showed a bimodal shape, with the minimum response below that of the water control (deterrent).

Fractionation of the hexane extract on a silica gel column resulted in four major lipid fractions. The extract and its fractions were all tested at 0.4 X. The chloroform fractions, C1 and C2, were almost as stimulatory as the original hexane extract, HEX (Figure 1). The combinations of C1 and C2 did not stimulate feeding stronger than its components. The acetone, A, and methanol, M, fractions were not different from water. In contrast, the hexane fraction, H, was feeding deterrent.

Extracts of tomato leaf surface with hexane and chloroform also stimulated feeding. Chloroform dewaxed leaves were less preferred than untreated leaves; however, hexane dewaxed leaves were equally acceptable as untreated leaves.

Thus tomato leaves contain feeding stimulants which belong to the neutral lipid group and may be found at the leaf surface. Tomato also contains feeding deterrents belonging to the group of hydrocarbons.

Insects and pyrrolizidine alkaloids

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Several insect groups have independently developed adaptations to utilize pyrrolizidine alkaloids (PAs) from plants. The phenomena involved are different from other insect plant relationships in various respects and thus provide some new general features. In a comparative view, this system could become a useful model for the study of coevolution.

To date, the Lepidoptera are the best studied group. While some of them obtain PAs because the larvae feed on suitable plants, others gather them only in the adult stage: e.g. Danainae and some Arctiidae are attracted to withered PA-containing plants, apply a PA-dissolving fluid onto the plant and reimbibe it (Boppré, 1978, 1981). Thus, the uptake of PAs is, in these cases, independent from assimilation of nutrition, and it therefore requires special sensory, physiological, ethological, and ecological adaptations.

Probably, the basic function of PAs in both plants and insects is primarily defensive. The insects store the toxic PAs (Edgar et al., 1979) and exhibit aposematic characters; many of them are known to be avoided by predators and/or involved in mimicry associations.

Another, presumably secondarily evolved and more striking function of PAs in insects is their use as precursors for male pheromones. From a variety of PAs with different molecular structures, obtained from a range of different plants, Danainae and Arctiidae synthesize three different dihydropyrrolizines (Schneider et al., 1975; Schneider et al., 1982), and Ithomiinae produce a lactone (Edgar et al., 1976b). These PA-derivatives are essential stimuli in close range sexual communication. Thus, taxonomically unrelated lepidopteran genera not only use identical PA derivatives for communicative purposes, but the reproductive success of the males depends on the ingestion of PAs. Other toxic plant metabolites are not known to be utilized for pheromone biosynthesis.

PAs have also been found to affect morphogenesis: In the genus *Creatonotos* (Arctiidae), the PAs ingested by larvae specifically regulate the growth of the an-

droconial organs, and also serve as precursors of the male scent (Schneider & Boppré, 1981; Schneider et al., 1982). Thus, a secondary plant substance specifically regulates morphogenesis of an organ which then releases a chemical signal synthesized from the same compound as the regulator. Again, there is no previous knowledge of a similar morphogenetic effect generated by a secondary plant substance.

The extensive investment involved in the above cases in both adapting to PA-plants in the course of evolution, as well as obtaining and utilizing PAs in the life of the individual, needs to be emphasized. The active search for and ingestion of PAs by adult insects independent of feeding behaviour requires to be considered in both the discussion of mimicry/automimicry and of sexual selection in the Lepidoptera.

Insect-PA relationships occur in several insect groups and in a variety of plant families (mainly Asteraceae, Boraginaceae, Fabaceae) which contain PAs of various chemical structures. By studying the insects' specificities for PA-perception, attraction, conversion, and utilisation it is hoped to shed light on the evolutionary strategies behind the various adaptations, which have occurred convergently, and thereby to understand the basic biological significance of PA-utilisation.

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Leaf surface chemicals of seedling sorghum and resistance to Locusta migratoria

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Seedling sorghum is unpalatable to acridids partly as a consequence of the release, by the chewing action of the insect, of HCN and phenolic acids from the bound forms in which they occur in the intact plant (Woodhead & Bernays, 1978). However, observations of third instar nymphs of *Locusta migratoria* on seedling sorghum (cv. 65D, Botswana) showed that, on first contact, 80% of the insects rejected the leaves at palpation. When the surface wax was removed from the leaves with $CHCl_3$, almost 90% of the insects observed palpated and then immediately tested the leaf.

The whole surface wax extract was very deterrent to *L. migratoria* and when fractionated by thin-layer chomatography revealed components similar to those in most plant waxes. In bioassays, the hydrocarbon and ester fractions were deterrent, and the fraction of highest polarity was the most active. This latter has been identified as consisting predominantly of *p*-hydroxybenzaldehyde (Woodhead et al., 1982). A content of authentic *p*-hydroxybenzaldehyde in dry matter of 5 g/kg was highly deterrent and concentrations in seedlings of 65D are over 10 g/kg (Woodhead, 1982).

Analysis of the esters showed one major (C_{24}) alcohol esterified to a range of fatty acids, more than 60% of which have chain length less than C_{18}. The hydrocarbons proved to be n-alkanes of predominantly odd C-number with more than 60% of chain length less than C_{26}. Synthetic esters of short chain acids ($C_{12,14}$) and the C_{24} alcohol were rejected by *L. migratoria* whereas long chain acid esters were palatable. Authentic n-alkanes of chain length C_{18}-C_{28} have been tested where commercially available. Preliminary results indicated that even C-number alkanes (uncommon in plant waxes) were palatable but odd C number, short chain ($C_{19,21,23}$) alkanes were not. Further work is in progress.

Surface waxes vary qualitatively and quantitatively with plant age and with cultivar. Alkanes and esters from seedling Indian cultivars IS1082 and CSH1 are not deterrent and have less than 5% of short-chain components. Similarly, older, palatable sorghums have major alkanes of C_{29} and C_{31} and acid components of esters of $C_{20,22,24}$ (Bianchi et al., 1978).

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Insect antifeedant compounds and other substances from Bupleurum fruticosum

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Large screening experiments show that a crude ether extract from *Bupleurum fruticosum* L. gives strongly positive antifeeding tests against several phytophagous insects: *Mythimna unipuncta, Leptinotarsa decemlineata,* and *Spodoptera littoralis.* Furocoumarins generally present in most *Umbelliferae* are well known as insect antifeedants (Muckensturm et al., 1981a). However no furocoumarin has been found to occur so far in the genus *Bupleurum.* In a preliminary communication (Muckensturm et al., 1981b) we assumed that phenylpropanoids are responsible for this repellency since methanol-water-chloroform partition followed by silicic acid chromatography furnished α-methoxy-estragol 1a (R = Me) and α-methoxy-methyleugenol 2a (R = Me). But gas chromatography and HPLC show clearly that these compounds appear only after methanol treatment. Whereas in the crude ether extract they are present as a series of fatty alcohol derivatives (R = long chain).

Capillary gas chromatography coupled with mass-spectrometry of the same methanol treated sample shows the presence of additional compounds:
— α-methoxy-elemicine 3a (R = Me);
— sesquiterpene hydrocarbons: copaene, bisabolene, ylangene, elemene;
— the presence of nonacosanone-10, estragole and monoterpenes (Carbonnier & Cauwet-Marc, 1979) is confirmed.

Reasonable amounts of these new α-methoxy compounds, for spectral comparison and biological tests, could be obtained by synthesis: reaction of vinyl magnesium bromide on the correctly substituted benzaldehyde followed by methylation.

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An investigation into the physiological actions of feeding deterrents

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Secondary plant substances are used extensively as one of the means whereby an insect recognizes a plant. In some cases such chemicals act as phagostimulants but in many they act as deterrents and inhibit feeding. One assumption is that deterrent substances are themselves toxic, or are associated with, or have similar characteristics to chemicals which are (a kind of mimicry). We are investigating the possible relationships between deterrency and toxicity by using *Locusta migratoria migratorioides,* and oligophagous species deterred by many plant secondary compounds and *Schistocerca gregaria,* a polyphagous species which tolerates or is even stimulated to feed by many of the same compounds.

Single injections either into the haemolymph or into the gut show that nicotine, azadirachtin, allylisothiocyanate and tomatine are significantly more toxic to *Locusta* than to *Schistocerca* whereas sinigrin, umbelliferone, salicin and canavanine are of low toxicity and show no interspecific differences. Tolerance to the compounds is greater following cannulation into the gut than injection into the haemolymph. This is a different spectrum of response to that of deterrence at the sensory level, where, with the exception of azadirachtin, all the compounds are more deterrent to *Locusta* than to *Schistocerca* (Haskell & Mordue (Luntz) 1969; Bernays & Chapman 1977; Bernays, unpublished observations).

The metabolism of several of these compounds by the insect is presently being followed using a variety of techniques. We have found that radiolabelled nicotine is excreted from the haemolymph at a significantly higher rate in *Schistocerca* than *Locusta.* High pressure liquid chromatography analysis is being used to monitor the contribution of different tissues and organs to the metabolism of nicotine. After cannulation of nicotine into the gut much is excreted unchanged, although low levels of cotinine can be detected. Azadirachtin, an extremely potent feeding deterrent (Haskell & Mordue (Luntz), 1969), has pronounced inhibitory effects on gut muscle contraction and prevents the passage of food through the gut. Further work on the mode of action and metabolic fate of these compounds is being carried out to un-

derstand in more detail the different feeding preferences of the two locust species.

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Olive chemical compounds influencing fecundity of Dacus oleae

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In summer the olive fly, dispersed throughout the region, returns into the olive-groves; this produces an increased number of catches in visual, attractant (Lupo, 1943) and pheromone traps. Also actual population density increases (Neuen-schwander & Michelakis, 1979). Simultaneously, in the female gonads, the number of developed eggs increases (Stravakis, 1973). Oviposition occurs a few days after emergence. Sometimes − in dry places − eggs are laid only at the end of the summer.

Such complex reproductive behaviour of *Dacus oleae* could be explained by considering that the host plant − at a particular ripening stage − gives off attractant and oogenesis stimulating compounds, while oviposition undergoes further stimuli.

Females avoid depositing in already infested fruits because they perceive substances (even volatile) from the lacerated tissues of attacked olives (Cirio, 1971; Girolami et al., 1981). When females are offered olives containing a part of already infested ones in the laboratory, fecundity will decrease exponentially and inversely to this ratio (unpublished data).

Recently, it has been shown that in olives, some substances are present which − sometimes as traces − stimulate oviposition on artificial beds. A synergistic activity has also been demonstrated between different olive fractions (Girolami et al., 1981).

The latest research results are set out concerning volatile substances collected (in a cold vacuum trap) from the oily fraction of olives. The quantity obtained from one single olive is sufficient to induce a good stimulation of oviposition on artificial beds in our usual laboratory tests.

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Analysis of gustatory activity using computer techniques

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Key-words: gustatory receptors, action potential analyses, chemoreception

The dose-response curves of 5 alkali chlorides were obtained from the tarsal D-hairs of 6-8 animals using 2-3 repetitions for each of 6 concentrations.
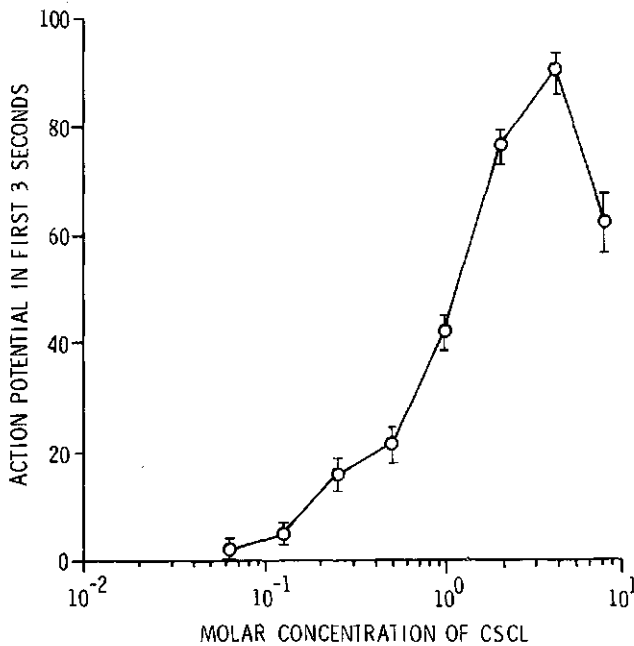
Fig. 1. Dose-response curve for the blowfly foretarsal salt receptor. Total number of action potentials in the first 3 s after onset of stimulation are plotted vs. molar concentration of cesium chloride. Data points ($\pm$ S.E.) are averaged responses from 8 animals, 10 receptors per animal, and 3 repetitions of each concentration.

As an example, Figure 1 illustrates the cesium chloride dose-response curve averaged from 8 animals with S.E. bars indicating total variability. Although two spike classes were seen, only the 'classical salt receptor' was studied in detail. One of the primary contributions of this investigation was the development of computer techniques to discriminate among different classes of spike shapes and to statistically analyze and graph the data.

More complex responses, e.g., the tobacco hornworm maxillary styloconica responses to leaves and leaf extracts, are currently under study using the computer analysis technique.

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Sensillum numbers and host-plant relations

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Orthopteroid insects have large numbers of chemoreceptors on the mouthparts and antennae; even in the smallest stages the chemosensitive neurones are numbered in thousands. These neurones are not sharply tuned to particular host plant chemicals and food selection is presumably based on the overall sensory input. These insects are generally polyphagous or graminivorous. In contrast, Hemipteroidea and Endopterygota have few chemoreceptors on the mouthparts, although adults of larger species may have large numbers of antennal receptors. A relatively large proportion of these insects are quite limited in their choice of host plant and in some cases this specificity is known to be associated with the presence of key chemicals to which some receptors are tuned.

It is suggested (1) that a reduction in the numbers of sensilla together with the development of tuned receptors facilitated the development of feeding specificity, (2) that this in turn facilitated diversification on different host plants, and (3) that large numbers of olfactory sensilla are present only in relatively large insects capable of reaching the source of an odour from some distance away (Chapman, 1982).

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Electroantennogram responses of the cereal aphid Sitobion avenae to plant volatile components

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Key-words: Sitobion avenae, olfactory receptors, chemoreception, plant odours, kairomones, virginoparae, oviparae

The reception of plant odours in the cereal aphid *Sitobion avenae* (F.), was studied by making use of the electroantennogram technique (see for methods Visser, 1979). An electroantennogram response (EAG) represents the summed receptor potentials of the olfactory receptor cells in the antenna in response to stimulation by
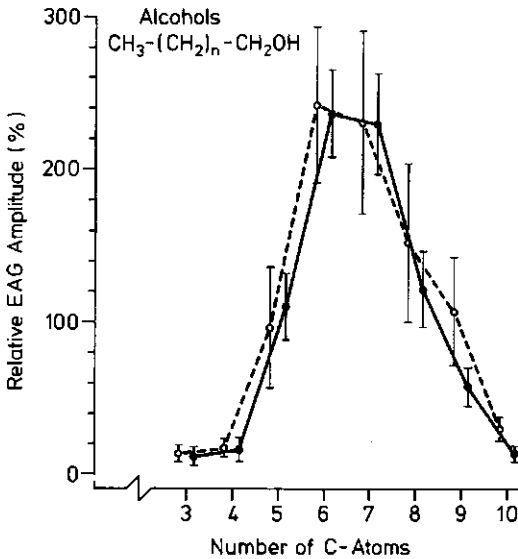
Fig. 1. Effectiveness of saturated alcohols in eliciting EAG responses of *Sitobion avenae*. Mean EAG responses as a percentage of the response to cis-3-hexen-1-ol. Broken lines: apterous virginoparae (n:10); solid lines: alate virginoparae (n:11). All compounds were diluted in paraffin oil at 10 μl/ml. Vertical lines indicate 95% confidence intervals.

Physiological and biochemical changes in three cultivars of chrysanthemum after feeding by Tetranychus urticae

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Physiological and biochemical changes in three cultivars of chrysanthemum after feeding by the two-spotted spidermite *Tetranychus urticae*, were studied under greenhouse conditions. The three cultivars, 'Super White', 'Dark Flamengo' and 'White Horim' differ in susceptibility to *T. urticae*.

The number of eggs per female three days after the start of oviposition as well as the population density after 5 weeks was slightly higher on 'White Horim' (moderately susceptible).

Chlorophyll content was reduced on all cultivars; the strongest reduction occurred on 'Dark Flamengo' (highly susceptible). Dry matter decreased from 11 to 19% in all three cultivars. It is possible that the dry matter content of infested leaves was dependent on the amount of stored starch. Significant differences in reducing sugar content after spider mite feeding occurred in all three cultivars. The sugar content was 2-3 times higher in the infested leaves; the highest increase occurring in the most susceptible cultivar. This may indicate that the mites modify the food plant quality to their own benefit. A strong decrease in photosynthetis activity and chlorophyll content was observed. The reaction of Super White was very interesting. The intensity of photosynthesis was three times lower in the damaged leaves as compared to the check plants, but chlorophyll content was only 18% lower. This may indicate a compensatory reaction by the damaged plants (see Kolodziej-Tomczyk et al., 1975; Poskuta et al., 1975 and Kolodziej-Tomczyk et al., 1979).

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Primary ultrastructural symptoms of mites' feeding in strawberry leaves

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The ultrastructural changes in strawberry leaves, *Fragaria grandiflora* Duch., cv. Georg Soltwedel, after infestation by the two-spotted spider mite, *Tetranychus urticae* (Koch), were investigated. Mature leaves from uninfested and infested (3, 6, 9 days) plants were fixed in glutaraldehyde, postfixed in OsO_4, dehydrated in ethanol and embedded in Epon 812. Ultrathin sections were stained with lead citrate and uranyl acetate before examination with an electron microscope.

Electron micrographs demonstrate that whereas the healthy cells possess well-developed plasmamembranes and submicroscopic particles, these structures show considerable instability and breakdown features in the infested tissues. Those damages are to be found even before the visual symtoms appear on the leaves. Micrographs indicate also that mite feeding may induce disorganization of the lamellar system of chloroplasts, whose arrangement is necessary for optimum phytosynthetic activity. In highly injured tissues misshapen cells contained homogenous protoplasts in which only pieces of necrotic chloroplasts were visible.

These results suggest that the chloroplasts should be considered as the primary candidate for mites' damage effect, and the morphological alterations within these structures may be regarded as one of the reasons of cell-death (see Kielkiewicz, 1980 and Szaniawski & Kielkiewicz, 1982).

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Electrophysiology of interaction between plant volatiles and sex attractants in several moth species

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Behavioural experiments have shown that in the moths *Antheraea polyphemus, Acrolepiopsis assectella* and *Yponomeuta cagnagellus*, the females' release of sex pheromones is stimulated by host plant odours.

In *Adoxophyes orana* and several species of *Yponomeuta*, electrophysiological experiments indicated that the olfactory response to sex attractants is modified by plant volatiles such as *trans*-2-hexanal, butanal, benzaldehyde, geraniol, nerol, eugenol and linalool. Most of these plant volatiles inhibit response of cells sensitive to sex attractants, but linalool highly activates cells in males of *Y. evonymellus* and in females of *Y. rorellus*. Although electroantennogram (EAG) experiments show that special receptors primarily responsible for plant volatile reception are present, experiments demonstrate that there is a direct interaction at the receptor level between plant odour and the response to a sex attractant.

To study these interactions in more detail, single sensillum recordings have been made using male and female antennae of *Agrotis segetum*. The results show that pheromone receptor cells can be activated by a number of plant volatiles. Apart from the cells responding to pheromone components, cells sensitive to plant odours can be found in the same sensillum. Gas chromatographic techniques have been used to determine the concentration of the odour stimuli actually applied on the antennal receptors during the recordings.

A re-appraisal of insect flight towards a distant point source of wind-borne odour

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This exhibit re-appraises the behavioural evidence concerning insect flight toward a point source of wind-borne odour in the light of meteorological information not yet considered in this context. The horizontal tracks of puffs of smoke from a generator in the open air were videorecorded and found to continue along nearly straight lines from the source for at least 25 m, while the shifting wind direction caused the plume formed by the succession of puffs to 'snake' to and fro. It is inferred from this and previous work by atmospheric physicists that within such a distance the wind will be aligned on the source of any wind-borne odour wherever the odour can be detected. This being so, a strategy of finding the odour source by flying roughly upwind on meeting the odour, but holding station against the wind with or without casting across it on losing the odour ('odour-modulated anemotaxis'), seems likely to be highly adaptive, whereas a strategy of flying along the plume ('odour-trail following') seems unlikely since it would often take the flier in 'wrong' directions and would be more disrupted by turbulence.

We conclude that the traditional image of insects flying along an odour plume lacks field credibility as well as experimental support, whereas odour-modulated anemotaxis appears to be a mechanism that is well adapted to a range of wind conditions. Whether it results in the flier following the plume continuously or meeting it only intermittently will depend on the rate of change of wind direction. When the wind direction is not constant the plume meanders and the insect might be in contact with the plume for only a fraction of the total time taken to reach the source. The present need is for further field testing of the theory of odour-modulated anemotaxis.

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Odour source finding behaviour of Delia brassicae in the field

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The cabbage root fly, *Delia brassicae* (Anthomyiidae) is a cosmopolitan pest, the larvae damaging roots of cruciferous crops. Gravid females of the right age and physiological condition respond clearly to the odour of the *Brassica* crop up to a distance of 15 m when kept in cages downwind of the crop (Hawkes, 1975). Hydrolyzed glucosinolates are strongly implicated as the main long-range guiding stimuli during the host finding process (Finch, 1980). However, detailed information about the various orientation mechanisms during the search for odour source in the field is still largely lacking.

Flies of the first generation were captured in a ploughed field by an arrangement of 25 Moericke-traps set 5 m apart in a square. The outermost 16 dishes, the 8 dishes in the second row and the single innermost dish captured on the average 15.4, 8.4 and 7.0 flies per dish per day, respectively. When each dish was equipped with a tube emanating allylisothiocyanate (ANC) the numbers of captured flies were on the average 29.3, 15.1 and 10.5, respectively. When only the inner 9 dishes had ANC tubes the catches were 19.5, 13.2 and 10.2 flies per dish per day, respectively. It is suggested that the flies' landing response to colour of the odourless dishes was intensified by the odour from the nearby ANC dishes.

In observations of the behaviour of single flies a wide odour plume was created by putting 10 ANC-containing glass tubes at 10 cm distances in a row at right angles to the prevailing wind; the upper edges of the tubes were flushed with the ground surface, thus being practically invisible. The area 50 cm wide downwind from the middle of the tube row was watched to count the number of flies landing within 0-150 cm (N1) or 150-200 cm (N2) downwind from the odour source. The observer was 5 m away from the area; wind was moderate (1-3 m/s). Each fly landing on the areas was captured for identification.

The flies seemed to fly short distances near the odour source, with stops of 5-50 s. Assuming constant step length one can calculate the average 'hop' of the flies from the ratio of landings to the two areas (N1/N2 = 92/42) by the formula: N1/N2 =

(X - 50)/50, which gives the value of ca. 160 cm. After flying past the odour source the flies mostly landed very soon, taking off again mostly in cross-wind and down-wind directions.

In wind-tunnel experiments Hawkes et al. (1978) observed also short upwind flights of ca. 1-2 m, while Finch (p.275) found that gravid females fly upwind even in the absence of host plant odours. In the anemotaxis of moths, zig-zagging in the odour plume is the standard manoeuvre, but for *Delia* and possibly other dipterans the orientation system seems to be quite different. More detailed field observations are needed to unravel the intricacies of odour-guided host plant search in *Delia*.

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The response of Scolytus scolytus to volatile host plant stimuli

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The elm bark beetles *Scolytus scolytus* F. and *Scolytus multistriatus* Marsh. are generally unable to feed and oviposit in the phloem of the stem and thick branches of healthy elms. However, they do feed and oviposit in such plant tissues if the tree has been physiologically weakened by mechanical damage, water stress or fungus infection. They also feed in the axils of 2 to 4 year-old twigs of healthy elms. Apparently there are resistance factors in the bark or phloem of thick branches or the trunk of healthy elms which are not yet operating in young twigs and which break down by a physiological decline of the tree.

These factors have not yet been identified. By preventing the beetles from twig feeding in the crowns of healthy elms one could possibly reduce the spread of dutch elm disease substantially.

To investigate the behavioural responses of the beetles to attractant or repellent host plant stimuli I record the behaviour of the walking insect simultaneously with two video cameras (entire test arena and close up) and analyse the behaviour with help of the slow motion and single frame facilities of the first video recorder. The Apple microcomputer system is used as eventrecorder and as a store and analysing device for the insects tracks. A simple and cheap bioassay arena, used only for a single test, allows the beetle to respond to volatiles with similar behaviour patterns as it displays in its natural environment on the bark of a host tree in response to entrance holes of other conspecific individuals.

The observed response behaviour to attractant stimuli is composed of orthokinetic, klinotactic and tropotactic elements. Not only the antennal clubs but also the maxillary palps play an important role as receptors in such a close range situation. The response pattern as well as the response intensity is highly individual among different beetles. The responsiveness of the test beetle population varies substantially and depends on temperature, humidity, light-intensity, circadian rhythms, age and previous experience. The response intensity in such a close range situation is much stronger if natural stimuli such as frass, phloem tissue or water are presented

than if artificial stimuli such as pentane extracts of frass or phloem tissue or pheromone blends or single components thereof are presented. Field trapping experiments corroborate these results.

The search for substances which could prevent the beetles from landing and feeding in the crowns of healthy elms will be continued.

Response of Trypodendron lineatum to ethanol production by its host tree, Picea excelsa

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The ambrosia beetle, *Trypodendron lineatum,* (Coleoptera: Scolytidae) attacks several resinous tree species, but *Picea excelsa* is the most heavily infested species.

We investigated the volatile products that attracted this beetle primarily to *P. excelsa.* Trees, that were pulled down recently, emitted many compounds which we were able to collect by condensation procedures and which we could identify as respectively α-pinene, β-pinene, p-cymene, α-phellandrene, terpinene and terpinolene.

Emission of ethanol starts about two and a half months after the cut-down of the trees. Ethanol production starts earlier in wood with small diameters, and also in wood which is pulled down during the summer season. Stripping of the trunks reduces the ethanol production considerably. Storage in a glade gives a delay of the production. The amount of ethanol produced is dependent on the type of wood; this is more important for wood which has not been stripped and is stored in the forest.

Ethanol is the primary attractant for *T. lineatum* and conditions the colonization of the host tree by this beetle. Primary attraction is followed by secondary attraction caused by a pheromone emitted by the female beetles. But the action of the female pheromone is rapidly overruled by some chemical substances produced by the male beetles. Both male and female *T. lineatum* beetles show almost the same response to ethanol.

From these experiences the following recommendations are suggested for the forest in the Belgian Ardennes:
- All felled wood must be stripped.
- All dead wood must be cleared from the forest as soon as possible, in particular between the months September and March.
- For monitoring of the beetle, traps with ethanol at a concentration of 20% in 300 ml should be suspended in the forests. Density of the traps: 2 per hectare in the first year, 1 in the second year (see Istace et al., 1982 and Magema & Severin, 1975).

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Oviposition stimulants for the carrot fly in the surface wax of carrot leaves

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The female carrot fly *Psila rosae*, (Diptera: Psilidae) is laying its eggs in the soil near its host plants: cultivated and wild Umbelliferae. Prior to oviposition the female is landing on potential host plants. On the host leaves a typical 'oviposition run' (average duration 50-80 s) is performed which is terminated when the female is descending along the leaf stem to the oviposition substrate (soil or artificial device). The host leaf surface contains compounds which can be extracted and which stimulate oviposition.

Raw extracts were prepared by dipping intact carrot leaves in CH_2Cl_2 twice for 30 s. This treatment does not disrupt the epidermal cells. Consequently this extract contains little or no compounds occurring within the leaf. The raw extract of 1.5 kg leaves was subject to two fractionations using silica gel columns and two different separations on a HPLC column with a gradual elution using solvents of increasing polarity. The activity of the fractions and subfractions was determined by applying amounts equivalent to the weight of a fresh leaf on artificial filter paper leaves. The number of eggs deposited under the treated leaves was compared with those under the solvent treated leaves (control). All the active fractions were analysed with a GLC-MS system and subject to further fractionation if necessary. In one of the two active silica gel fractions we identified the two propenylbenzenes trans methylisoeugenol and trans (iso) asarone. The other more active fraction yielded 4 subfractions with significant activity. The active compounds identified so far were the polyacetylene falcarindiol and the linear furanocoumarins bergapten and xanthotoxin. The purified raw extract of one carrot leaf was estimated to contain about 25 µg falcarindiol. This amount of the pure compound significantly stimulated oviposition. Bergapten has proven to be active at 100 µg per artificial leaf. Other furanocoumarins (both angular and linear) known to occur in several species of Umbelliferae were found to be active at 1 mg per leaf. We are trying to duplicate the activity of the raw extract using equivalent amounts of the pure compounds in singular form and in an artificial mixture.

All three groups of compounds (propenylbenzenes, furanocoumarins and poly-acetylenes) are typical allelochemicals of the Umbelliferae which together, do only occur in this family. Thus perception of these compounds by the carrot fly does account for the observed host plant selection by the female. The importance of these compounds is further underlined by the fact that they inhibit the growth of bacteria, fungi and non-adapted phytophagous caterpillars.

Guerin et al., (p.95) have shown that the propenylbenzenes are perceived by the antennae. It seems possible that the receptors for the other compounds may be located on the tarsi.

Allopatric speciation in the small ermine moth Yponomeuta padellus

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In western Europe, the small ermine moth *Yponomeuta padellus* L. (Lepidóptera, Yponomeutidae) can be found on various plant species, of which *Crataegus monogyna, Prunus spinosa*, and *P. domestica* are its most common host plants. Occasionally, however, *Prunus cerasifera* and *Amelanchier* sp. are infested too. In Finland, on the contrary, *Crataegus monogyna* and *Prunus spinosa* are almost completely absent and replaced by *Sorbus aucuparia* as host plant of *Y. padellus*. The occurrence of *Y. padellus* on *Sorbus aucuparia* in that country is the more remarkable, as infestations of this plant species in other parts of Europe are rare or even not known.

Therefore, oviposition choice and food preference experiments were carried out in order to compare the population of *Y. padellus* on *Sorbus* in Finland with various populations of the species on *Crataegus* and *Pr. spinosa* in the Netherlands and W. Germany, respectively.

Table 1. Oviposition choice in *Yponomeuta padellus*. Ovipositing females from populations on *Crataegus monogyna* (the Netherlands) and *Sorbus aucuparia* (Finland) show a strong predilection for their own host plant.

Origin	Oviposition (%) on	
	Crataegus	*Sorbus*
Crataegus	91 (64)[1]	9 (6)
Sorbus	15 (5)	85 (29)

1. Number of egg batches.

Results of oviposition choice experiments show a strong predilection of the females for their own host plant (Table 1), while food preference experiments resulted in a higher survival rate and/or pupal weight when feeding the larvae on their own food plant(s).

Consequently, it is concluded that by means of allopatric speciation a new host race on *Sorbus aucuparia* in Finland has evolved.

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Allozyme characterisation of sibling species and host races in two families of Lepidoptera

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The zymogram method (electrophoretic separation of enzymes and subsequent specific staining) is an outstanding technique for measuring hybridisation between 2 genetically different populations, as hybrids normally show codominant expression.

The species-complex related to the small ermine moth, *Yponomeuta padellus* (Yponomeutidae) is composed of 5 species that are morphologically almost indistinguishable. Allozymically, however, each species is characterised by at least one diagnostic enzyme. No hybridisation was found in natural populations after analysis of some 7 500 individuals. Contrary to expectation F_1 hybrids from artificial crosses between the 3 most biochemically related species usually show repression of the maternal information. *Y. padellus* is associated with various food plants in Western Europe. Sympatric populations on 2 host plants sometimes show differences in their allozyme frequencies that are far too great to be due to chance alone.

For example, in Domat (Switzerland) *Y. padellus* occurs on *Crataegus monogyna* and *Prunus domestica* (Figure 1). The frequency distribution of allozymes of hydroxybutyrate dehydrogenase indicates the absence of allozyme 102 from *P. domestica*, whereas its frequency on *Crataegus* is 0.20 (Figure 1). It is highly improbable ($P < 0.001$) that allele 102 will not be detected at all in the sample from *P. domestica* (sample size 50) assuming that individuals on the 2 host plants comprise a panmictic unit. Analogous situations occur in the malic enzyme ($P < 0.01$) (Figure 1) and malate dehydrogenase ($P < 0.05$) loci.

Host-specific selection is not very likely and therefore the 2 populations belong to different mating groups and can be termed host races (sensu Mayr). Such host races may have higher genetic distances than populations on the same host plant hundreds of kilometers apart. Analogous situations in the family Nepticulidae (leafminers) are at present under study. In one case several species described from *Rubus, Fragaria, Geum* and *Agrimonia* turn out to be one species (*Stigmella aurella*), which possibly hybridises in certain localities with its sibling species *S. spendidissimella.*

Y. padellus on Crataegus

100 102	98 100	100 106
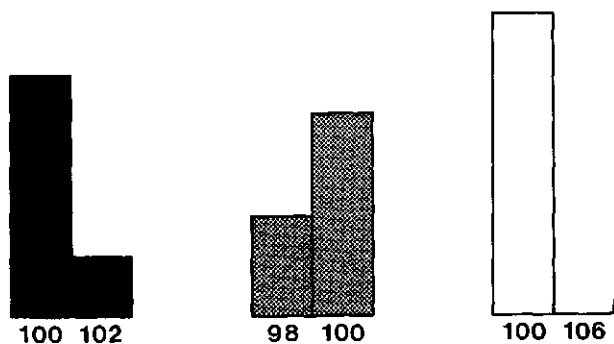
Y. padellus on P. domestica

100 102	98 100	100 106
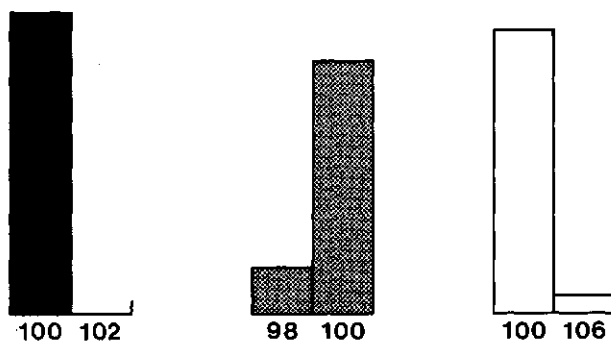
Fig. 1. Allozyme frequencies of *Yponomeuta padellus* on *Crataegus* (above) and *Prunus domestica* (below) in Domat (Switzerland). Enzymes indicated are hydroxybutyrate dehydrogenase (black bars), malic enzyme (grey bars) and malate dehydrogenase (white bars).

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Electrical recording of aphid penetration

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Electrical recording has been introduced by McLean & Kinsey (1967) and modified from an AC to a DC device in this study. The method uses the aphid as part of an electrical circuit: an adjustable voltage (E) is supplied to the feeding substrate (Fs), plant or artificial diet; the penetrating aphid makes connection to an amplifier (A) input. A high value resistor (R) connects this input to ground (Figure 1). In case of no penetration the signal gives a base line. As the aphid penetrates, a series of complex signal patterns can be recorded. The signal has been described as composed of the main patterns, A, B, C and D (Tjallingii, 1978).

The signal appears to have two different electrical origins: resistance fluctuation and electromotive force (e.m.f.). A special amplifier is able to record the e.m.f. component selectively. Our usual device records mainly resistance fluctuation with relative low e.m.f. amplitudes in addition.

The most remarkable e.m.f. component is the potential drop (= voltage drop, see Tjallingii, 1978) during pattern C (Figure 2). It appears to have a rather constant value of -100 mV to -150 mV. Penetration of artificial diets do not show these potential drops. However, penetrations of isolated plant cell protoplasts in a solution behind a Parafilm ® membrane show similar potential drops as in intact plant tissue. In plant penetrations pattern D often occurs at a constant potential drop level.

These results indicate that the phenomenon is associated with plants or plant cell protoplasts. The membrane potentials of plant cells are from -100 mV to -200 mV.
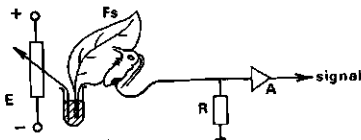
Figure 1. Scheme of the main parts of the electrical recording set-up. See text.

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Figure 2. Potential drop during penetration of a plant or a plant cell protoplast. Horizontal bar: 5 s, vertical: 100 mV.

The phloem has a cellular character. So our hypothesis is that the potential drops are momentary penetrations of cell membranes at regular intervals on the way of the stylets to the phloem and often ending inside a phloem cell.

It has to be mentioned that pattern D also occurs without a constant potential drop.

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The probing behaviour of Rhopalosiphum maidis

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Field observations record that the corn leaf aphid, *Rhopalosiphum maidis* only occurs in small numbers on sorghum seedlings (Woodhead et al., 1980). In order to determine possible chemical and physical deterrents, an investigation into the performance and probing behaviour of *R. maidis* has been performed on different growth stages of three *Sorghum bicolor* varieties; CSH 1,IS 2663 and IS 2501c. Also an investigation has been started to determine factors which influence orientation of stylets in leaf tissue.

The performance of the aphid is influenced by the age of plant. On all three varieties significantly fewer offspring ($P \leq 0.05$) were produced by aphids caged on seedlings, (growth stage 1, Vanderlip 1972), than those on older plants, (growth stage 3). On seedlings of variety IS 2663, larval development time was significantly longer ($P \leq 0.05$) and adult weight lower than on older plants.

The probing behaviour of *R. maidis* is also affected by the age of the host plant. Probes left in leaf tissue were predominantly intercellular and in older tissue, the majority of probes were traced to the phloem. In seedling leaf tissues, probes with two or more branches were frequently found and fewer probes terminated in the phloem. As a result of branching, total probe lenghts were longer. Branched probes can be associated with mechanical barriers such as the lignified tissues of the xylem, although the branches in seedling tissues terminated in mesophyll and bundle sheath regions.

The probing behaviour of *R. maidis* in older leaf tissues suggests that probing is less random than in young tissue. If the aphid is guided towards the phloem, directional responses must be influenced by components of the intercellular space. To alter the composition of the intercellular spaces, sucrose was applied to the adaxial surface of a mature sorghum leaf. Aphids were allowed to probe from the abaxial surface for 1 hour. More branched probes were found in tissue treated with sucrose and probes could be traced to the adaxial epidermis, although none were found at this site in the control.

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Cereal aphid honeydew production

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Two major cereal aphid pests *Sitobion avenae* and *Rhopalosiphum padi* may cause considerable economic damage to cereal crops. *S. avenae* causes significant reductions in grain yield (14% by weight) (Wratten, 1975) and grain quality (Wratten & Redhead, 1976). *R. padi* is a vector of barley yellow dwarf virus. In addition, accumulation of honeydew on the leaves and developing grain of *Triticum aestivum* may reduce photosynthesis and encourage the growth of sooty moulds such as *Cladosporium* spp. and *Alternaria* spp.

The aim of this project is to investigate the factors influencing the qualitative and quantitative aspects of honeydew production.

Aphid honeydew production was monitored throughout individual lifespans on vernalized and non-vernalized seedlings (G.S. 12-13) (Tottman & Makepeace, 1979) c.v. Flanders, and correlated with aphid performance. A significant difference ($P \leq 0.05$) occurs in the number of days to maturity on vernalized and non-vernalized seedlings. Vernalization lengthens the larval development time. Significant differences in honeydew production occurred within treatments as well as between vernalized and non-vernalized seedlings. Honeydew production therefore shows much variability. The majority of honeydew produced occurred during the reproductive phase of the aphid. Mean honeydew production per aphid is 5.04 mg for *S. avenae*, and 3.33 mg for *R. padi*.

Amino-acid analysis of honeydew has revealed fifteen amino acids. Carbohydrate analysis shows the presence of ten sugars. Amounts of the four principal sugars, sucrose, glucose, fructose and melezitose vary during the lifespans, and indicate considerable differences between the two aphid metabolisms.

Collection of honeydew from *S. avenae* on field plots having different fertilizer treatments shows a significant increase ($P \leq 0.05$) in honeydew production on underfertilized plots of c.v. Hobbit. No difference was found with c.v. Flanders. Honeydew production was not affected by overfertilization.

S. avenae is generally believed to be a phloem feeder. It's preferred feeding site is

the rachis and glumes of the developing grains. An investigation has been started to determine which tissue is being tapped for nutrients. Initial results have shown that the majority of probes found in leaf tissue are unbranched and terminate in the phloem. Probes found in glume tissue were predominantly branched, frequently passing beyond the lower epidermis.

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The movements of apterous Myzus persicae on sugar beet

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Myzus persicae (Sulz.), the green peach aphid, is an important virus vector in the sugar beet root crop (*Beta vulgaris*). Current techniques for determining aphicide spray advisability and timing are not efficient, and the success of control is poor (Jepson & Green, in press). The need to improve control stimulated an investigation of the factors influencing secondary dispersal of virus diseases following primary infection by migrant alate *M. persicae.* The first part of this study examined the aphid—host plant relationship and in particular those factors that influence apterous aphid dispersiveness.

Observations in controlled environment and field conditions (in preparation) have shown that apterous *M. persicae* on sugar beet plants make repeated leaf-to-leaf movements. These movements are related to particular stages of leaf development, suggesting that the suitability of the leaves for aphid colonisation changes during growth. Important nutrients and phagostimulants are depressed in concentration during a hiatus in translocate flow between the 'import' and 'export' phases of leaf development and it is during this phase that the aphids tend to disperse and colonise other leaves. The leaves that the aphids settle on are either in the youngest age classes ($\sim$0-15% of final leaf area) when essential amino acids and sugars are in highest concentrations, or in the later stages of growth ($\sim$80% of final leaf area) when maximum rates of photosynthesis occur.

The dynamic nature of *M. persicae* colonisation of sugar beet has important implications for virus spread. The frequent movements by apterae support the hypothesis that these morphs are as likely to disperse virus from initial foci of infection as alatae. This mechanism of secondary virus dispersal also explains the pattern of radial spread of virus infection from initial foci. Further studies identified a period when the aphid population declines, probably due to a decline in the nutritional suitability of the whole plant; no secondary virus dispersal is therefore likely to occur after this period.

This work may help to explain the variation in rate and extent of virus dispersal between fields. The construction of a simulation model of secondary virus dispersal will provide a tool for the development of improved control tactics.

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Inter- and intraspecific variation in the performance of eight species from the genus Aphis

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The genus *Aphis* (Homoptera: Aphidoideae) contains both specialist 'insignificant' aphids and some notorious pests. The performance of five specialist species, *A. sambuci, A. corniella, A. hederae, A. grossulariae* and *A. epilobii* was compared with three pests. *A. fabae* and *A. gossypii* both extremely polyphagous, very successful but different in adult size and *A. pomi* which has a restricted host range. Experiments were conducted at two temperatures, 15°C and 20°C. At 15°C larval development time was an average of 29% longer; mean adult weight, 28% heavier: the number of embryos per milligram adult weight, 28% fewer: the intrinsic rate of increase 23% lower and fecundity 11% lower. *A. fabae* showed greatest response to temperature difference followed by *A. hederae, A. sambuci, A. grossulariae, A. gossypii, A. pomi, A. epilobii* and *A. corniella.*

For the same adult weight, pest species have a higher fecundity than non-pest species, a shorter development time, slightly more embryos per unit adult weight and a higher intrinsic rate of increase. Whereas non-pest species have a declining intrinsic rate of increase as adult size increases, the reverse is true for pest species. Pests are not only animals in the 'wrong place' but their biological performance characteristics considerably enhance their capacity for increases in numbers far beyond that displayed by species on plants of no economic importance.

Examinations of the influences of hooked epidermal hairs of french beans (Phaseolus vulgaris) on the pea aphid, Acyrthosiphon pisum

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Key-words: Acyrthosiphon pisum, french beans, trichomes, plant resistance

Pea aphids, *Acyrthosiphon pisum* (Harris), settle spontaneously on french beans (*Phaseolus vulgaris*) and show probing behaviour. However, subsequently a considerable proportion of aphids leaves the plant. Aphids remaining on the plant become impaled on the hooked epidermal plant-hairs and die within one week (at 20°C), although experiments indicate that french beans are physiologically suitable as a host plant for *A. pisum*. The hairs are scattered over almost the entire surface of french bean plants and hinder aphids in leaving the plant. If aphids are stimulated by an approaching hot needle to quickly leave the plant, many of them are prevented from doing so. Experiments show that the percentage of aphids successfully escaping from the plant by drop-response is negatively correlated with hair density and length of settling time. However, aphids settling on a hairless plant like broad bean (*Vicia faba*) are able to escape when irritated.

Mortality and migration of *A. pisum* on french beans are dependent on hair density which varies with the bean variety. Many more aphids are able to leave the sparsely haired variety 'Zuckerperl Perfektion' (upper leaf surface 150 hairs/cm^2, lower leaf surface 300 hairs/cm^2) than the densely haired variety 'Molina' (upper leaf surface 570 hairs/cm^2, lower leaf surface 1400 hairs/cm^2). Consequently the mortality of *A. pisum* is much higher on 'Molina' than on 'Zuckerperl Perfektion'. Those aphids remaining on the plants after being trapped by the hooks die within one week, but there are no differences at all between the longevities of these aphids on both varieties.

In order to examine differences in physiological suitability of both varieties for *A. pisum* the fecundity of settled aphids was measured until they died. There are no significant differences in fecundity at 20°C on both varieties, with 7.1 larvae per female (range 0-15) on 'Zuckerperl Perfektion' and 6.1 larvae per female (range 0-14) on 'Molina', respectively. Given the choice to settle either on the sparsely or on the densely haired variety of bean in a mixed-culture, aphids will settle on both varieties in the same proportion within the first hours of the experiment. However, three days later about 80% of the aphids were found to have accumulated on the densely haired variety.

Susceptibility of Tilia species to the aphid Eucallipterus tiliae

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A honeydew problem occurs most years in Britain on lime trees growing in ave-
nues and as amenity trees as a result of infestations of lime aphids on the foliage.
The most frequently occurring lime in Britain with this problem is the common lime
Tilia × *europaea* and most of the studies on the lime aphid have been made using
this host. The caucasian lime *T. euchlora* has the reputation of being resistant; other
species in the genus *Tilia* have not been compared for resistance nor has the reason
been investigated. The species differ in either having a smooth ventral surface to the
leaves or various degrees of pubescence caused by hair arrangements on the epider-
mis.

Kidd (1976a) has shown that on *T.* × *europaea* the early instars are obliged to
feed on the minor leaf veins as their stylets are incapable of reaching the phloem of
lateral or main veins. The older aphids can, and tend to, feed on the larger veins.

The pubescence of certain white limes like *T. petiolaris* is very dense on the un-
derside but less dense on the main veins. Most (82%) adult females could feed on
these but the few new-born nymphs that were produced could not reach the tissue
and all starved on account of a barrier of interdigitating stellate hairs (Figure 1A).
When the pubescence was scraped off and fourth-instar virginoparae were confined
by clip cages to the cleaned area, they readily matured and gave birth to young. By
using van Emden's (1969) method of measuring the mean relative growth rate, the
young aphids on cleaned *T. petiolaris* leaves had a similar performance to those
aphids reared on the freqently infested and native host *T. platyphyllos.*

The silver lime *T. tomentosa* has a similar leaf pubescence. In Turkey where it is
native, another closely related aphid, that has not yet been described (V.F. Eastop,
personal communication) lives on these leaves. The adults have a rostrum 20-40 μm
larger than the British *E. tiliae,* and as the stellate hair bases of *T. tomentosa* are 25-
35 μm long this aphid appears to have overcome the barrier by its longer reach.

Another pubescent species *T. maximowicziana* also failed to support aphids. Of
83 individual fourth-instar aphids tested there were none surviving after four days.
Unlike *T. petiolaris,* however, the removal of the pubescence in this species did not
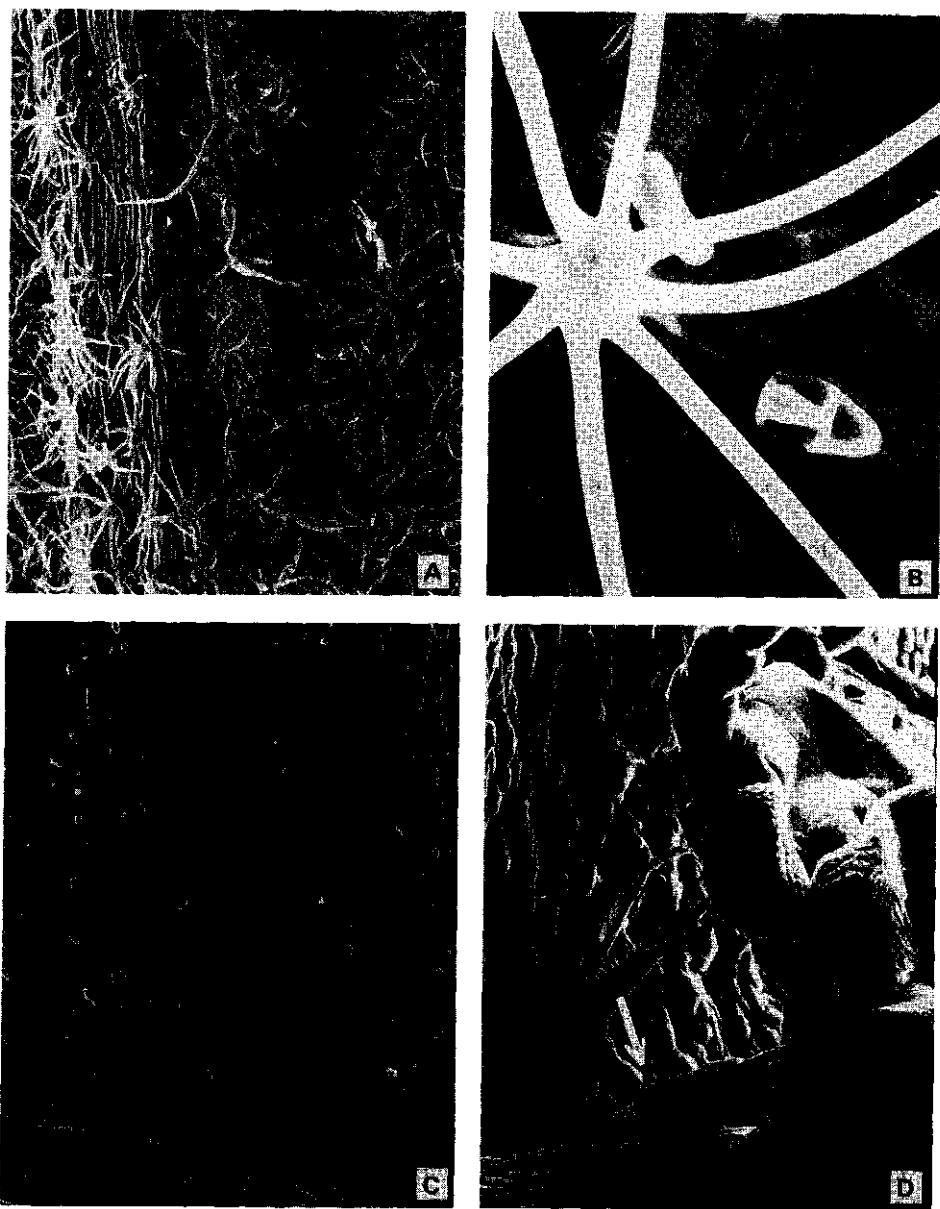
Fig. 1. Scanning electron micrographs. A — ventral leaf surface near main vein of *Tilia petiolaris* showing the barrier of dense stellate hairs (× 88); B — ventral leaf surface of *T. maximowicziana* showing glands on a minor vein beneath the level of stellate hairs (× 680); C — ventral leaf surface of *T. euchlora* with glands distributed along the veins (× 41); D — rostrum of *Eucallipterus tiliae* in the feeding position on the side of a main vein of *Tilia cordata*.

remove the feeding inhibitor. Examination of *T. maximowicziana* leaf surfaces under a scanning electron microscope revealed that between the coating of stellate hairs there are small glandular prominences lying on the leaf cuticle (Figure 1B).

Feeding tests by individually confining 80 fourth-instar virginoparae on the dorsal or ventral surfaces to each of the smooth leaved species *T. euchlora* and *T. mongolica* showed a similar inhibiting property. In all cases the aphids died within five days. Scanning electron microscope examination of the ventral leaf surface shows (Figure 1C) a presence of similar glands to those found on *T. maximowicziana*. The distribution of the glandular prominences over the minor veins and along the sides of the major veins in *T. euchlora* coincides with the feeding sites selected by aphids feeding on *T. × europaea* (Kidd, 1976b) where glands are sparse (Figure 1D).

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Some ecological consequences of genetic variability in the polyphagous aphid Myzus persicae

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Insects use different means to survive and successfully reproduce in a more or less heterogenous and unpredictable environment. With respect to their food plant, they may be highly specialized and well adapted to a single food source or at the other extreme they may be generalists and utilize a wide variety of food items. Such a highly polyphagous insect is the aphid *Myzus persicae*, (Homoptera, Aphididae), which is impressive in its physiological ability to grow and reproduce on over 100 plant species.

In the present study we took samples of *M. persicae* populations in four agricultural areas differing mainly in the predominant food plant for the aphid (sugar beet, potato, vegetables, and a mixed crop growing area). A permanent culture of each clone sampled was kept in the laboratory on rape leaves and the population increase was measured during 12 days for each clone on sugar beet and potatoes. The data indicate a wide genetic variability of food plant adaptation for the population on both host plants, with highly significant differences in population increase, fecundity and length of reproduction between single clones.

Ecological consequences of this genetic variability are at present under study. First results from populations sampled in autumn 1980 and during 1981 indicate, that subpopulations of the aphid, which are genetically adapted to their respective host plant, develop during the vegetative period. The ecological unit of host plant adaptation appears to be at least on a per field level irrespective of other food plants in the same area, since subpopulations on a sugar beet field and a potato field, which were in the mixed crop area only a few hundred meters apart, proved to be well adapted to their respective food plant.

However, subpopulations appear to be temporary and their identity is lost when aphids leave sugar beets and potatoes and infest turnip (*Brassica rapa*) fields in August. Life cycle differences between clones, which were analyzed in the laboratory under short day conditions, also contribute to the wide genetic variability of the population. Holocyclic and anholocyclic subpopulations in general show the same

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pattern of host plant adaptation as mentioned above for the whole population. However, holocyclic clones at least on potatoes have a significantly higher population increase than anholocyclic clones.

Actography of Myzus persicae on susceptible or resistant peach seedlings

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The feeding behaviour of *M. persicae* (Sulz.) was studied by actography on two peach varieties, one susceptible and the other with an intermediate degree of resistance to the aphid. Aphids were forced to feed on certain places: apical or mature leaves, apical stems, but some of them could chose their feeding sites freely. The experiments lasted from 4 to 16 h and the speed of the strip chart recorder was 12 cm/h. Two classes of data were surveyed:
— total duration of electrical zero 'salivation time' and 'ingestion time' (McLean & Kinsey, 1967)
— some parameters of the first feeding period with 'ingestion'
• first period of 'salivation' not followed by 'ingestion' (S_1),
• first period of 'salivation' followed by 'ingestion' ($S.I_1$),
• first period of 'ingestion' (I_1),
• the sum of each of these parameters, for example $I = I_1 + I_2 \ldots$

The results were analysed by the Kruskal and Wallis test:
— On the basis of all the data, the aphid preferences are the following: apical susceptible stem, apical resistant stem, apical susceptible leaf, apical resistant leaf, mature leaves. The preference for apical susceptible stem, and the repellency of mature leaves are very strong.
— The data suggest that resistance factor(s) may be present:
• before the discovery of a feeding site:
• during 'salivation' not followed by 'ingestion' on mature leaves;
• perhaps during 'salivation' followed by 'ingestion'. The mean values of SI_1 are greater on apical stems than on apical leaves. They are also greater on apical stems of resistant seedlings than on apical stems of susceptible seedlings.
• at the feeding site on apical resistant stems, on apical susceptible and resistant leaves, on mature leaves.

Histological studies would explain (1) whether the aphid on apical resistant stems and apical susceptible or resistant leaves cannot reach a suitable feeding site or if the phloem is unsuitable (2) the part of physical factors in peach resistance.

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Holcus, Holcaphis and food quality

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In the grass *Holcus mollis* L. seasonal peaks in soluble nitrogen levels related to spring and autumn growth periods and anthesis positively influence numbers of phloem feeders. (McNeill & Southwood, 1978). However, although the aphid *Holcaphis holci* Hardy, a specialist feeder on *Holcus,* is undoubtedly affected by both between year and seasonal variations in levels of soluble nitrogen (McNeill, unpublished data), there is evidence that it can alter these levels to its own advantage.

Holcaphis produces a 'gall', affected shoots being stunted with shorter, wider leaves. The rate of leaf production is increased but the duration of these leaves is reduced. This alters the leaf age structure, giving shoots with a very young mean leaf age.

It also could be expected to stimulate nitrogen transport to and from the growing point, thereby increasing the amount available to the phloem feeding aphids. Preliminary amino-acid analyses seem to confirm this; total amino-acid, and in particular amide content being increased in the leaves, stems and apices of infected shoots. It is known that amides represent important forms in which nitrogen is transported from one part of the plant to another (Salisbury & Ross, 1978). In addition, van Emden & Bashford (1971) have already shown that amide has a positive effect on the relative growth rates of the aphids *Brevicoryne brassicae* and *Myzus persicae.*

It appears that *Holcaphis,* faced with seasonal fluctuations in its food quality, alters its host's phenology and lives in a perpetual 'Spring flush' of nitrogen.

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Behaviour and biology of two aphid species related to gall morphogenesis

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Key-words: Chermes species, galls, *Picea excelsa*

Chermes abietis L. and *Chermes strobilobius* Kalt. (Adelgidae, Aphidoidea) cause the well-known 'pineapple galls' of the Norway spruce (*Picea excelsa* L.) (Figure 1). In autumn, stemmothers move to the young twigs and, after selecting a suitable site, insert their stylets, never leaving the spot again throughout the rest of their life. The
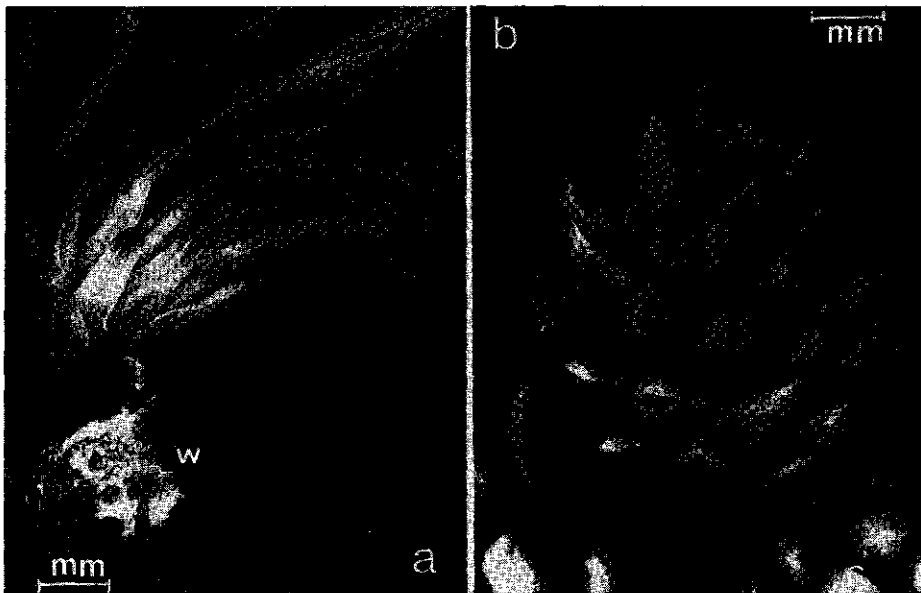
Fig. 1. Young galls of *Chermes abietis* (a) and of *Chermes strobilobius* (b) at the time when gallicolae migrate between the modified needles. W: eggs.
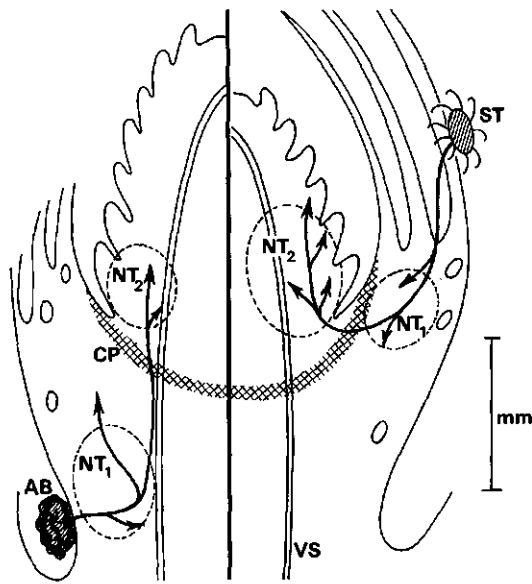
Fig. 2. Schematic diagram of a bud of *Picea excelsa* at the end of the winter, showing the fixation and the feeding sites of 2 adelgids: *Chermes strobilobius* (ST) and *Chermes abietis* (AB).
NT1: nutritive tissue of the insect in autumn
NT2: localisation of the nutritive tissue in spring
CP: collenchyma plate
VS: vascular elements

stylets are always inserted intercellularly. The stylet tracks reveal the feeding behaviour of the aphids. The insect's feeding on the young bud modifies bud growth and development to produce small shelters between the modified hypertrophied needles, where the offspring find food. Both aphids, *Ch. abietis* and *Ch. strobilobius* (Figure 1) can attack the bud of the same host plant at the same moment.

The two kinds of plant responses are directly related to the selective nutritional behaviour of the two aphid species: choice of a particular type of bud such as small, lateral, or strong, terminal and choice of a particular point of attack (Figure 2). There are also slight differences in the development of both aphid species.

All these characteristics, leading to a specific localisation of the attack in space and time, have allowed each species to develop its specific ecological niche and are responsible for the morphological and biological differences between the two galls. During early cecidogenesis, *Ch. strobilobius* escapes easily and permanently from the normal development pattern of the host organ, whereas *Ch. abietis* is always in competition with normal growth and is subject to various resistance phenomena such as hairs, lignin, tannins.

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Variation in virulence in populations of the brown planthopper, Nilaparvata lugens in Asia

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Nilaparvata lugens (Stål) is effectively specific to cultivars and wild species of rice (*Oryza*) in Asia where it is a major pest. Populations have been isolated at the International Rice Research Institute (IRRI), Philippines, which show distinct patterns of virulence on rice varieties which incorporate different genes for resistance. These have been thought to be genetically homogeneous and termed Biotypes 1, 2 and 3 with the ability to survive respectively on cultivars with no gene for resistance (eg. TN1), gene Bph 1 (eg. Mudgo), and gene Bph 2 (eg. ASD7).

The inbred biotype cultures at IRRI show great individual variability in virulence (Claridge & Den Hollander, 1980) and virulence seems to be inherited by a system of polygenes and not by simple major genes (Den Hollander & Pathak, 1981). Selection experiments on appropriate cultivars over eleven generations showed that it is possible to convert one so-called biotype into a population with virulence characters of another.

An allopatric population from Queensland, Australia, behaved to TN1, regarded as universally susceptible, as if it were resistant. It was cultured in Cardiff on the French variety Delta. Selection experiments were made on this population. After great problems of low survival in the early generations, a population was selected able to survive and reproduce on TN1. It resembled Philippine 'biotype 1' in virulence characteristics. In other respects the Queensland population showed considerable genetic differentiation from Philippine ones (Claridge et al., 1982).

Field populations of *N. lugens* were sampled from five different cultivars and from wild rice (*O. rufipogon*) in Sri Lanka in July, 1980. The populations were tested for virulence in the laboratory in Cardiff on the varieties from which they were collected and on the standards — 'TN1', 'Mudgo' and 'ASD7'. Most populations showed distinct patterns of virulence and evidence of close adaptation to their own varieties. One even showed lack of virulence on 'TN1'. Thus, even within a small geographical area, there is good evidence for local variation in virulence.

We conclude that *N. lugens* populations are highly labile for virulence characteristics and susceptible to rapid adaptation by natural selection. The biotype terminology is thus not appropriate to this species.

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Morphological variations among three biotypes of the rice brown planthopper Nilaparvata lugens in the Philippines

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Occurrence and evolution of prolific biotypes of the rice brown planthopper (BPH) *Nilaparvata lugens* (Stål) are constant threats to the stability of pest-resistancet varieties presently under cultivation (Pathak & Saxena, 1980). At present, identification of these biotypes is based principally on differential reactions of host rice varieties to the pest or on host-mediated, differential behavioural and physiological responses of the pest. A morphological basis for identification of brown planthopper biotypes remains to be established. In many organisms, changes in ecological and physiological traits of the species are frequently followed by subtle changes in morphological characteristics (Bay-Bienko, 1958). We, therefore, made
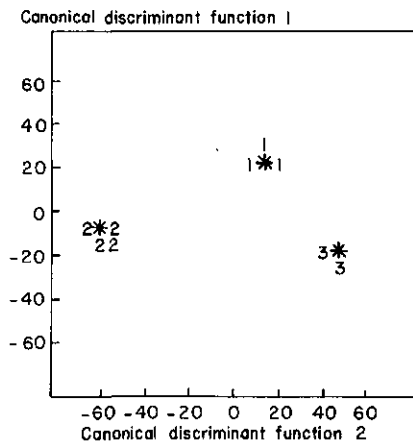
Fig. 1. Discriminant scores of three biotypes of *Nilaparvata lugens* based on rostral, leg, and antennal characters of brachypterous females. The numbers indicate biotype designation; the asterisk (*) indicates a group centroid.

an in-depth evaluation of morphological and morphometric differences among populations of brown planthopper Biotypes 1, 2 and 3 maintained as stock cultures at IRRI for several years, concentrating on body parts possessing receptors used for host plant discrimination, such as rostrum, legs and antennae.

One hundred adults from each population of Biotypes 1, 2 and 3 maintained on TN1, Mudgo and ASD7 rice varieties, respectively, were prepared for morphological examination as follows: (1) boil in 95% ethanol for ca 10 min; (2) macerate in lukewarm 10% NaOH for 10-15 min; (3) wash in 95% ethanol and boil for 15-20 min in chloral-phenol; (4) clear in creosote for 10 min; (5) mount body parts on glass microslides using Hoyer's medium. Antennae were also mounted in glycerol medium on slides so that they could be moved freely during microscopic examination. Camera lucida drawings of selected structures were made at $20 \times$ objective of a phase contrast microscope. More than 100 morphological characters of the rostrum, including mandibular stylets, legs and antennae were measured and evaluated. Characters were examined separately in both sexes and their morphs, i.e., macropterous ♂, macropterous ♀, brachypterous ♂, and brachypterous ♀.

Multiple discriminant analysis during stepwise selection through Wilk's specification indicated distinct segregation of the three biotypes. The characters of the rostrum, legs and antennae common to both sexes and their respective morphs contributed to the separation of biotypes. The scatter diagrams based on computed discriminant scores of the three biotypes strongly revealed a high degree of segregation, thus, classifying them as distinct populations (Figure 1). The hoppers classified on leg and antennal characters exhibited a 100% probability of correct morphological identification of the three brown planthopper biotypes. The details are being published elsewhere.

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Relation between population density of Phyllocnistis suffusella and genetical and environmental characteristics of poplars

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The author studied the population density of caterpillars of *Phyllocnistis suffusella* Z. (Lepidoptera), a poplar-leafminer. The aims were to prove whether there are differences according to the genetical variations among poplars, the age of the tree and the soil characteristics and to propose hypotheses to explain these differences.

During 5 years 13 poplar clones were studied. The trees were planted at random (one-tree-plot); there were 7 clones of *Populus deltoïdes* × *nigra* (PDN), 3 of *P. trichocarpa* (PT) and 2 of *P. trichocarpa* × *deltoïdes* (PTD). Most trees were new clones of the Instituut voor Populierenteelt, Geraardsbergen (B). Significant differences in population density were found between the groups (PDN > PT > PTD), within the groups PDN and PT, but not with PTD (the studied clones have the same parents). Population density was highest on poorly drained and loamy soil, but was lower on sandy soil.

In view of the disposition of the proof plantations the most probable hypothesis is that soil and clones cause differences in caterpillar mortality. The leaf composition is the most probable link between the insect and the studied environmental factors.

Poplar sensitivity to Phratora (Phyllodecta) vitellinae: analysis by different methods

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Key-words: Phratora vitellinae, plant resistance, poplar clones, antifeedants, feeding stimulants, extraction, fractionation

The sensitivity of different *Populus* clones to a chrysomelid defoliator beetle, *Phratora (= Phyllodecta) vitellinae* L., has been analysed by choice experiments:
– in experimental plots in the field, as already has been done with poplars and other insects by Arru (1973), and Lapietra (1973);
– on experimental cuttings in a greenhouse (Finet & Grégoire, 1981);
– on leaf discs in the laboratory, as already has been done, for instance, by Bongers (1970) with *Leptinotarsa decemlineata* Say.
 The following clones were compared: 'Ghoy 1', 'Ghoy' (in the nursery only), 'Gaver', 'Robusta vert', 'Fritzy Pauley', 'Columbia River', 'Unal' and 'Beaupré' (in the nursery only). 'Ghoy 1', an indigenous *Populus nigra*, is by far the most resistant. On the opposite, the *P. trichocarpa X deltoïdes* clones ('Unal' and 'Beaupré') have been globally the most intensively attacked. The *P. deltoides X nigra* ('Ghoy', 'Gaver' and 'Robusta vert') seem mildly sensitive. The *P. trichocarpa* ('Fritzy Pauley' and 'Columbia River') are rather resistant, except 'Columbia River' which, in the nursery at least, is much attacked.
 The results obtained with the three methods were similar, but low differences in sensitivity do not stay necessarily from one experimental mode to another. The great, constant resistance of 'Ghoy 1' and the general great susceptibility of 'Beaupré' led us to compare them in a chemical study using methods often described, for instance by Hsiao & Fraenkel (1968). Extracts were made from leaves and submitted to the insects on small TLC cellulose plates. These experiments look promising to discover the substances responsible for the resistance or susceptibility of poplars to *Ph. vitellinae.*

Acknowledgments

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Host plant changes in several populations of Caryedon serratus

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Caryedon serratus (OL) (Coleoptera) is a generalist Bruchidae of African origin that is able to reproduce on the pods of six bushy Caesalpinioideae, one Mimosoidea and one cultivated plant, the groundnut *Arachis hypogea* (Faboidea), (Decelle, 1981). The females lay their eggs on *Arachis* pods once the plant have been pulled up and left to dry in the fields. We have analysed the ability of *C. serratus* to change its host by studying the reproduction and development of populations from different geographical origins when placed in the presence of pods from one host bush *Bruchus rufescens* or with *A. hypogea* pods.

A population originating from Niamey (Niger) was obtained from pods of *B. rufescens* and was always reared on the same host. In this region, there are no groundnut cultures. In the laboratory, when inseminated females were in the presence of *B. rufescens*, oviposition began on the first day of the experiment and 98% of the eggs were deposited on the pods. In the presence of *A. hypogea*, oviposition began more slowly and only 78% of the eggs were placed on the pods, while the others were scattered around the rearing boxes. The fecundity ($m = 253.2 \pm 3.2$) with *B. rufescens* was significantly higher than in the presence of *A. hypogea* ($m = 135.5 \pm 27.9$).

In the Maradi region (Niger), *C. serratus* has access to either *B. rufescens* or groundnut pods that have been left to dry. The larval development of this population can occur in groundnut seeds. In the laboratory, *B. rufescens* always had a higher stimulating effect on oviposition than *A. hypogea* ($m = 210.7 \pm 16.4$ with *B. rufescens*, $m = 148.5 \pm 11.1$ with *A. hypogea*).

A population found in stocks in the Bouenza region (Congo) was also examined. This population was collected in an equatorial zone where the wild host plants of *C. serratus* are absent. In the presence of *B. rufescens*, only a few females deposited eggs on the pods, 95% of the eggs were scattered around the rearing boxes. In this population fecundities ($m = 267.7 \pm 28.9$) were more important in the presence of *A. hypogea* than in the presence of *B. rufescens* ($m = 194.5 \pm 19.2$).

Males and females of the three populations can be crossed and their hybrids are fertile. When there is oviposition, larvae penetrate the seeds and can develop, whatever the population (mortality 27-35% in the two species of seeds, without overpopulation).

The population of Sahelian region can meet with the two species (*B. rufescens* and *A. hypogea*), and the females are sensitive to a wide variety of stimuli which allows them to deposit their eggs on the two types of pods. However, the stimulating effect of *B. rufescens* pods is always much greater. Contrary to different insects (Smith et al., 1979; Wasserman, 1981), it does not seem that larval conditioning influences adult ovipositional preference. The populations from Congo, where *B. rufescens* is absent, have probably adapted themselves to *A. hypogea* over several generations and have thus become more dependent on that plant for their reproduction. Their larvae remain generalist and can develop in seeds of *B. rufescens* when oviposition occurs on this plant.

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Effects of insects on the population dynamics of plants in a Mexican tropical rain forest

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This poster describes the effects of herbivores on seedling populations of six typical and contrasting tree species of tropical South-East Mexico. The study is based on observations in permanent quadrats. A structural analysis of the seedling communities showed that the seedling foliage is presented to the herbivores as localized patches of palatable or unpalatable resources. Of the variety of types of damage detected, insects were the herbivores responsible in all cases. For each plant species there was a particular type of damage. The results indicate that up to 60% of the individuals of each seedling population was damaged. From these damaged individuals less than 25% of leaf tissue was removed. An analysis of individual leaves showed a tendency of decreased survival with the increment of feeding by insects. Thus it appears that even minor levels of damage by insects may alter the delicate metabolic balance of seedlings on the forest floor.

Modification of insect-plant relations by plant stress

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Slight changes in the balance between the insect and its host can produce marked fluctuations in insect populations. Such changes can result from shifts in plant metabolism that occur with normal developmental processes or environmental stresses. Initial studies have been conducted with three types of stress factors, that is, air pollution, disease, and salt stress, as part of a series of investigations planned to examine the relationships between the response of insects to stress-induced host changes and their feeding habits and reproductive strategies.

Laboratory studies with sulfur dioxide showed that this common air pollutant induced changes in soybean, *Glycine max* L., that resulted in increased growth, rate of development, fecundity, and feeding preference of the Mexican bean beetle (MBB), *Epilachna varivestis* Mulsant; similar but much less pronounced effects were observed with pinto bean, *Phaseolus vulgaris* L., as the host. In a field test with soybeans, beetle fecundity and larval growth were greater on plants fumigated intermittently with SO_2 than on control plants. After less than one generation, beetle populations and feeding damage were both about 1.5 times greater on exposed plants than on control plants. In chamber studies, fumigation of soybean with SO_2 did not affect the success of the two-spotted spider mite, *Tetranychus urticae* Koch.

In experiments with a bacterial disease, infection of red kidney bean by the common blight, *Xanthomonas phaseoli*, had no apparent effect on the development time, growth, or mortality of either the southern armyworm, *Spodoptera eridania* (Cramer), or the MBB. However, the fecundity of the MBB was significantly higher when females were reared on infected leaves.

The effect of salt stress on the success of the two-spotted spider mite on soybean is being investigated. The rate of population growth of the mites was greater on stressed plants in two tests using either severely stressed stage V2-V3 plants or mildly stressed stage V5-V6 plants.

An insufficient number of stress-plant-insect examples has been examined to permit generalizations to be made, but clearly the success of plant-feeding arthropods

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can be greatly affected by this interaction. The experiments also suggest the possibility of such interactions having a greater effect on insects marginally adapted to a host, and more such examples need to be included in future investigations.

Effect of fire and drought on seed production in Banksia attenuata and on its seed predator Alphitopis nivea in western Australia

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The seeds of the small tree, *Banksia attenuata* R.Br. (Proteaceae) from south-west Australia are eaten by larvae of the weevil *Alphitopis nivea* Pascoe (Curculionidae). The weevil larvae complete their year long development inside the fruit, consuming the contents of three or four follicles. Each follicle has one or two seeds (Scott & Black, 1981). In some years, larvae are extracted from the *Banksia* fruits and eaten by the cockatoos, *Calyptohynchus funereus latirostris* Carnaby (Psittacidae). Also in some years, parts of the approximately 300 ha study area were burnt by wildfires. In a five year study of this site there was considerable variation between years for the percentage of follicles damaged by weevils. Damage ranged from 10% to 90%. Part of this variation was due to trees producing up to four to six times the number of follicles two years after some years with high rainfall or fire. Fire stops the plant's reproduction for two years and consequently prevents a generation of insects. In years of abundant follicle production, the weevils were present in similar numbers in burnt and unburnt areas but a greater proportion of follicles escaped attack in burnt areas. More weevil larvae in the burnt areas were attacked by cockatoos possibly because the birds are attracted to areas of high fruit production (Scott & Black, 1981). This study shows the importance of infrequently occurring environmental factors such as fire. These incidents make it difficult to predict the levels of damage caused by insects to the seeds of *Banksia attenuata* based on a few years data.

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Some effects of pollution on roadside fauna

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Most studies on the flora and fauna of roadside habitats have been concerned with effects of salt on roadside plants or accumulation of lead in roadside animals.

There have been few studies on the distribution and abundance of fauna in these habitats. However, large populations of herbivorous insects have been found on roadside shrubs, sometimes resulting in complete defoliation. Plants beside roads have higher levels of nitrogen than plants growing further from roadsides. Oxides of nitrogen in exhaust gases may increase the availability of nitrogen to roadside plants or stress, for example from de-icing salt, may also serve to raise nitrogen levels. Higher nitrogen levels in roadside plants may be responsible for increased herbivore
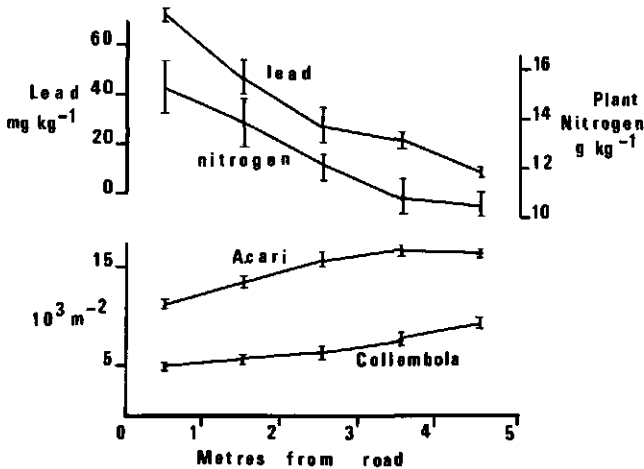
Fig. 1. The change in numbers of Collembola and Acari, soil lead levels and total plant nitrogen along a 5-metre transect beside a road. (Bars represent standard errors.)

populations (Port & Thompson, 1980) and might also be expected to affect soil animal numbers.

Williamson & Evans (1973) found no significant decrease in numbers of soil animals at roadside sites with high lead concentrations, in fact some groups showed increased abundance. However, they were comparing different sites.

We have assessed the distribution of soil fauna in relation to pollutants within sites using short transects at right angles to each side of the road. The numbers of more abundant groups, Collembola and Acari, were significantly lower nearer to the road. Soil lead levels and plant nitrogen content were both highest at points nearest the road (Figure 1). Animal numbers showed significant negative correlations with soil lead levels (Collembola, $r = 0.83$; Acari, $r = 0.649$). We conclude that animals with different feeding strategies are affected in different ways by the wide range of pollutants in roadside habitats.

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Foraging behaviour of Rhagoletis pomonella: a model for fruit parasites

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Current foraging models are simplistic and unlikely to accurately predict the foraging behaviour of animals in complex environments (Oaten, 1977). Of particular importance is the lack of attention devoted to search dynamics and neglect of factors affecting encounter rates between foragers and their resources (Roitberg, 1981).

We are currently studying, through field tests and simulation modelling techniques, the foraging behaviour of *Rhagoletis pomonella*, a tephritid fly that oviposits in and whose larvae are endoparasites (Price, 1977) of *Crataegus oxyacantha* (Rosaceae), as a model for parasitic insects. In this paper, we provide a general framework for foraging studies in which we define *R. pomonella* search dynamics and host encounter rates through elucidation of the following:

— Quantification of *C. oxyacantha* patch structure at three *R. pomonella* foraging levels, that is, tree, fruit cluster, and individual fruit.

— Reactive volume. Detailed field observations show that reactive distance by *R. pomonella* females depends upon relative position of individual flies to fruit. For example, flies positioned 15 cm below a fruit cluster locate that cluster with 80% success, whereas only 45% of flies locate that same cluster when 15 cm above it (Roitberg, unpub. data).

— Quantification of search paths of flies. Field observations show that flies display predictable search movements following presentation of various host fruit stimuli (Roitberg et al., 1982).

— Quantification of spatial and host visitation memory of individual flies. Flies are able to return to sites of successful foraging following forays away from those sites. In addition, fly foraging behaviour is influenced by visits to at least the previous five fruit hosts (Roitberg, 1981).

Integration of the aforementioned parameters into a stochastic, simulation model is currently being employed to realistically describe both *C. oxyacantha* location and encounter by *R. pomonella* in nature (Elkinton et al., in prep.). We suggest that similar approaches to studies of other parasite-host or predator-prey systems will

provide appropriate information from which to evaluate predictions from contemporary foraging theory.

Acknowledgments

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Interference among pheromone traps for the ambrosia beetles Trypodendron species

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Barrier traps baited with the aggregation pheromone lineatin, in combination with ethanol and α-pinene were used to investigate the effect of trap spacing upon catches of the ambrosia beetles *Trypodendron lineatum*, *T. domesticum* and *T. signatum*, in the Black Forest during the spring of 1981. Groups of three traps forming equilateral triangles the sides of which measured 10, 20, 30 or 40 m were set up, with three replicates per treatment. Considering all species together, the average number of beetles caught per trap increased significantly from 13.4 to 24.8 with the distance among traps, between 10 and 40 m.

Thus, phenomena of interference occurred among lineatin baited traps at distances larger than previously known (about 10 m). Analysing each species separately, the percentages in the total catches of both *T. domesticum* and *T. signatum* which are hardwood pests, increased with the distance among traps, while *T. lineatum* that attacks conifers, followed an inverse trend. Trap spacing did not affect the sex ratios observed: 1 ♂ : 0.7 ♀ for *T. lineatum* and *T. signatum*. For *T. domesticum* the most frequent value found was 1 ♂ : 0.4 ♀.

The results are discussed in connection with the different patterns of aggregation behaviour known for the three species from laboratory bioassays and field trails. Only *T. lineatum* should respond positively to α-pinene, this substance having a repellent effect upon the two other species. Different rates of dispersion of the volatiles used, lineatin, α-pinene and ethanol, should partially explain the findings. It is emphasised that the shape and dimensions of the plume of odours formed result from the micrometeorological conditions registered under the forest canopy, which in turn depend upon the topography. To some extent, this fact may impair comparisons among results.

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