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Proceedings of the 6th International Symposium on Insect – Plant Relationships (PAU 1986)

V. Labeyrie, G. Fabres
and D. Lachaise (editors)

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TOWARDS A SYNTHETIC APPROACH TO INSECT-PLANT RELATIONSHIPS

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In underlining the principal importance of the study of relations between insects and their host plants, for the elaboration of crop protection methods, "**The Insect-Plant relationships Symposia**" have brought agricultural entomology out of a cul-de-sac. They have diffused the coevolutionary approach, suggested by G. Fraenkel's article on the "**Raison d'être of secondary substances in plants**" (Fraenkel, 1959).

In the 20 years that have elapsed since the first symposium, numerous contributions have defined the influence of such substances upon insect attraction, feeding, assimilation, growth and reproduction. Many works concerning sensory physiology, physiology of nutrition, endrocrinology and physiology of reproduction have stressed the importance of plant characteristics - particularly chemical traits - in the modulation of insects' activities. For example, J. Kennedy (1961) highlighted the influence of trophic relations on migration and dispersal.

A precious lesson to be drawn from the whole of these works is the impossibility of defining - through simple parameters - insects' main activities, like longevity, time of growth, fecundity, dispersal, searching capacity, ... even when the physical conditions of the habitat have been stabilized (Labeyrie, 1970). It is strange to note that even today many mathematical patterns of population dynamics still use a fixed parameter to characterize fecundity, the intrinsic rate of natural increase, r_m (Labeyrie, 1970).

Biochemical studies of the allelochemical substances of plants have given rise to new possibilities of intervention, bringing along a new generation of insecticides. K. Slàma (1969), having discovered that plants can contain chemicals with insect hormone activity, Jan de Wilde (1971) has even proposed using plant substances to disturb insects' endocrine regulation and cycles.

At the same time, resistance factors have been brought out and have helped to lay the basis for selection of resistant cultivars.

Unfortunately, and even though plant chemists and insect physiologists have joined in their efforts, the lack of plant physiologists has not allowed us, up to now, to measure to what extent the influence of the plant could vary during its growth and according to ecological conditions. In many entomological works, the plant is considered as a homogeneous material. However, as early as 1951, in the 9th International Congress of Entomology, V. Dethier underlined that "**the plant is rather an heterogeneous chemical environment**" (Dethier, 1978). The attendance of

plant physiologists at this 6th International Symposium must help to set up a dialogue which, I hope, will grow into a habit.

Maybe, then, we entomologists, shall realize that damage caused by insects are not necessarily of great consequence for all kinds of plants (Labeyrie & Hossaert, 1985). In particular, we will probably be led to examine the reality of the selective pressures exerted by the destruction of the reproductive organs of perennial plants. Thus, the influence of the destruction of fruit and seeds on the evolution of the number of ovules per fruit and the number of fruits per plant, seem to me of no use for perennial plants, for which sexual reproduction only occasionally participates in the keeping up and renewal of the population. So, some speeches about coevolution often become the expression of an excessive romanticism.

Progressively, in the course of the recent symposia, the concept of population has directed an increasing number of contributions. The study of genetic variability in the relations between insects and plants, did not remain only a tool for the selection of resistant cultivars, but became also - as Vincent Dethier requested in 1978 at the 4th International Symposium in Slough (Dethier, 1978) - a means to understand the complexity of situations observed in the field. At the same time, awareness of genetic variability enables us to improve our physiological and biochemical studies.

A twofold movement has therefore developed during the last twenty years: the passage from the study of the insect to the study of polymorphic insect populations; and the enlargement of the study of the influence of plants upon insects from simple trophic control to the whole of their biology. So, the evaluation of the influence of plants, not only implies the analysis of their energetic content, but also research on the importance of the information issuing from the plants and their habitats, upon genesis and regulation of the sundry activities of the larva as well as the adult.

A global approach, rehabilitating the notion of what is called (Stearns, 1976) "**a life history strategy or tactic**", should have followed logically. Unfortunately, a partitioning tendency still slows down the movement. With the wish of improving their studies - and this is very creditable -some entomologists have often completely separated the analysis of different functions. The animal has become lost in an assemblage of independent activities, each of which was used with delight by proponents of modelling and theorizing, in order to define particular adaptive strategies (Gould & Lewontin, 1979). So, there have arisen studies concerning feeding strategy, sexual strategy, egg-laying strategy... with, in each case, a panglossian admiration for the value of the adaptive features put forward. **The unity of the insect is too often forgotten; the insect becomes a self-service super-market of strategies.**

During the same time, and acting in concert with the above tendency, the stress was laid on the energetic aspect of biocenotic relations. Starting from the statement of the importance of energetic transfers in trophic relations, everything has been evaluated according to its energetic

supply, and the value of behaviours has been estimated from their energetic costs. So, entomological studies, made from an ecological point of view, sometimes happen to be reduced to research on the energetic bill of the whole insect's activities. The entomologist thus becomes an accountant defining the adequacy of activities according to the balance-sheet. With the same point of view, some evolutionary ecologists (Colley et al., 1985) set up energetic relations to explain plants' adaptive reactions to destruction by insects, with a mechanist conception (since..., because...). Any consideration of the importance of the informational content of insect populations and of their biocenosis, is, in this way, eliminated.

This double reductionism, which emphasizes exclusively the energetic aspect, and sections the insect, splitting it into sectors of independent activities - sometimes arbitrarily defined - p**revents the examination of the relations between plants and insects in their whole complexity.**

In order to restore the genuine dimensions of insect-plant relationships, in introducing a sector missing in the previous meetings, the problems of pollination will be approached during this symposium. Maybe this confrontation will help to understand that manichean divisions between useful and noxious insects are not necessarily appropriate (Labeyrie & Hossaert, 1985). To give back to the insect its unity, to discuss the relations between polymorphic populations of plants and insects, to estimate the relative importance of the various kinds of relations between not less than two populations, such are the necessities required by the huge progress and the remarkable deepening of our sectorial knowledge of the insect. So, **the time has come - without slackening the intensity of detailed study - to gather the pieces and to work out the mosaic.**

It would be dangerous to idealize the relations between plants and insects, and to believe that coadaptation can be perfect. F. Jacob (1981) explained why evolution could only be as adjusted as **tinkering** is. I add, since these relations are not binary, but form a web of interactions, that this tinkering can only be the result of historical and provisional compromises between all the adaptations necessary to the survival of the plant in a particular ecosystem.

However, any endeavour of biocenotic integration would be incomplete and would lose its efficiency if biocenosis were handled like abstract entities without a spatio-temporal structure. The Earth is heterogeneous and mans' activities do not necessarily have a homogenizing effect. Consequently, every habitat has its own spatio-temporal characteristics, which modulate the relations between plants and insects. Now, in this field, we are quite behind. W.G. Wellington et al. (1984) pointed out, for instance, our ignorance of the effect of wind movements inside the low troposphere upon distribution of insects.

Because all biocenotic phenomena we are able to put forward, contain, at the same time, a part of universality and a part of contingency, crop protection is necessarily a local and circumstantial application of ecological knowledge (Labeyrie, 1977). So, we must be very careful in our deductions and express always contingent recommendations.

The day will never come when a handbook dealing with crop protection gives recipes suitable for the whole world.

Through these symposia on Insect-Plant Relationships, the last 20 years have allowed us to get into new and often unsuspected worlds. Our symposia have undoubtedly been profitable to applied entomology as well as to biological knowledge as a whole.

I am convinced that twenty years from now, the next evaluation will be still more surprising. The tools supplied by physics, chemistry and molecular biology multiply our possibilities for investigation, but the quality of results does not depend only on capabilities of tools. Never will the importance of hypotheses be devaluated, never will agronomists take the place of researchers.

To those who could be inclined to look upon the insect as out-of-date research material, and to consider entomology as an old-fashioned activity, we should reply like H. Fabre - **the genial experimenter** - as Darwin called him -"**The insect helps us to decipher just a little the most obscure book of all, the book of ourselves**" (Fabre, 1910).

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INSECT HORMONES AND BIOANALOGUES IN PLANTS

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The pharmacologically active drugs and toxins of plant origin have been known since the time of Aristotle. At the beginning of this century it has been also recognized that plants may contain quite specific animal products, such as are animal hormones. Some hormones were discovered in plants by chance or were accidentally obtained after screenings of plant extracts for various other activities. Only rarely a hormone was found in plants by purpose, usually in a search for developmental syndromes caused by certain fodder plants in the domestic animals.

There are many biological as well as chemical features that are common to animal and plant cells (especially cytochemical, anatomical, metabolic, biogenetic, see King, 1962). However, the nervous and neuroendocrine systems of higher invertebrates and of vertebrate animals have no direct functional counterpart in the plant kingdom. Thus, one could reasonably argue that the presence of animal hormones in plants might be a fortuitous combination of events having no causal evolutionary grounding. Alternatively, animal hormones belong to the biologically most effective compounds ever known. Their presence in plants, just like the presence of certain other pharmacologically active secondary plant subtances, may be thus an outcome of natural selection, which has undoubtedly modulated the interactions between plants and their animal feeders in the course of their coevolution.

The diffusible tissue factors of plants (plant hormones) have no direct endocrine functions in the animals, although some of them are often reported as a cause of diverse pharmacological side effects. The hormones of all animals show a common phylogenetic origin, which can be derived from neurochemical regulatory substances of the nerve cells in Coelenterata and worms. Some of them became successively separated from the nerve system and were produced by specialized endrocrine glands (see schematic outline in Fig. 1). The chemical structure of animal hormones is mainly derived from two biogenetically distinct categories: a) Derivatives of the aromatic amino acids, i.e. biogenic amines, peptides and proteins which are mainly used as neurohormones, and b) Isoprenoids, usually steroids, serving the role of the subordinated peripheral hormone (for review see Barrington, 1980 and Slama, 1982). The first reports related to the presence of animal homones in plants came from the studies on human steroid estrogen hormones. The history of this finding shows striking similarities with the more recent findings related to insect hormones. It may thus appear quite useful to mention a few points from the estrogen story here (for review see Slàma,

1980).

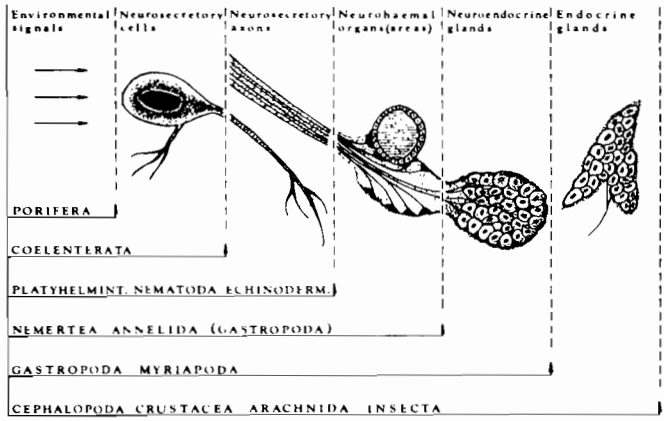


Figure 1. Evolution and diversification of the neuroendrocrine system of invertebrate animals (see Slàma, 1982).

1. Estrogenic hormones in plants

The search for a pharmocologically active natural product is always stimulated by availability of a suitable bioassay. After 1920, there appeared a biological assay for estrogenic hormones, known as Allen-Doisy test. And, still before structural elucidation of the estrogenic hormones, various authors observed surprisingly that positive Allen-Doisy tests on ovariectomized animals could be obtained with the lipid extracts from certain plants. Later, when the structures of steroidal estrogens became known, the active principles of the plant extracts were again reinvestigated. It was confirmed that the pollen grains, flowers and fruits of some plants indeed contained the true steroidal estrogenic hormones. The story became more interesting when further screenings of plant extracts revealed the presence of other estrogenically active compounds whose chemical structure and biogenetic origin substantially differed from that of the true steroidal estrogens. These compounds - phytoestrogens - were real pharmacobiological but not chemical mimics of the estrogens. Originally they were mostly found in the group of isoflavones (see Fig. 2). Further progress in this hormonomimetic research was made when it was determined that a sickness and infertility of sheep grazing on certain clover pastures in Australia were due to hyperestrogenic syndromes caused by a phytoestrogen coumestrol. Later it turned out that some fodder plants, especially Leguminous, could indeed produce such adverse effects in the domestic animals. The effects were usually due to the presence of benzofurocoumarin compounds, coumestrol, trifoliol, psoralidin and others (Fig. 2). Among further ecologically important phytoestrogen we may include a cyclic lactone zearalenone. It is produced by certain lower plants (**Giberella**). In domestic animals which would feed on spoiled grain, zearalenone produces serious hyperestrogenic syndromes associated with

infertility or decreased milk and meat production. There exist more phytoestrogen, such as miroestrol (see Fig. 2), which is a very potent estrogenic mimic isolated from a rejuvenating drug from certain Asiatic Leguminous plants. There are also several estrogenically active plant-borne stilbene derivatives, diterpenic compounds and other secondary plant chemicals that are believed to be estrogenic (for review Slàma, 1980).

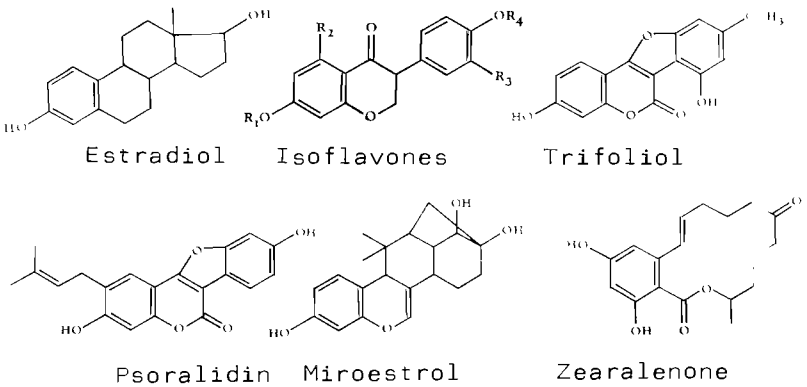


Figure 2. Structures of estradiol and several pharmacobiological mimics of estrogens isolated from plants (from Slàma, 1980).

The apparent structural heterogeneity of the phytoestrogen molecule brings about the problem of specificity of the biological assays. We could see on the examples in Fig. 2 that estrogenic assays selectively discriminate between chemical structures of the tested compounds. They give positive response only to molecules possessing in this case a more polar, keto, hydroxy or methoxy groups at exactly the same spatial and dimensional locations as in the molecule of estradiol or estrone. This imperative structural condition of all estrogenically active compounds has been tested and confirmed by X-ray crystallographic measurements in a number of natural or synthetic estrogen mimics.

2. The juvenile hormone of insects (JH)

In analogy with phytoestrogen, the history of insect hormones in plants is also related to a suitable bioassay. In this case it is the assay for JH elaborated by Williams in 1956. Soon after the assay became available, Schneiderman and his co-workers (Schneiderman et al.,1960) found JH-active lipid extracts in various microorganisms and in higher plants. The first JH-active substance of known chemical structure was an isoprenoid alcohol farnesol, isolated by Schmialek in 1961 from the excrements of the meal worm and from yeast (see Fig. 3). Farnesol was previously known as a common constituent of various plant oils.

Further presence of JH activity in plants has been accidentally found in American paper products and in the wood of the Canadian balsam fir by Slama and Williams in 1965. The active material, "paper factor" was

subsequently identified by Bowers as an alicyclic sesquiterpenoid ester called juvabione (Fig. 3). The wood of certain evergreen trees contains several JH-mimics related to juvabione. In addition to JH-I to JH-III (which have been isolated from insect body and are assumed to be identical with the JH of insects), there are some other JH-mimics of plant origin. These include, for example, sesamin from sesame oil, echinolone from American coneflower or juvocimene from sweet basil (see fig. 3). For references and more details see (Slàma, 1969, 1979, 1985; Slama et al., 1974; Williams, 1970). It is rather curious to realize that we know only a few JH mimics from plants in comparison with more than 4000 so far known synthetic analogues with JH activity (Slàma, 1985). However, the importance of the identified plant products may be stressed out by the fact that the knowledge of each of the compounds listed in Fig. 3 led always to subsequent synthesis of hundreds of its derivatives and so increased our knowledge concerning structure-activity relationships among these compounds (review in Henrick, 1982; Slàma, 1985; Slàma et al., 1974).

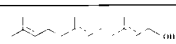
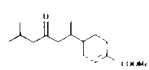
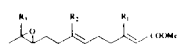
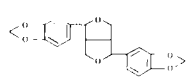
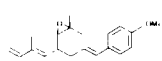
Structure	Radicals, notes	Source
	farnesol	yeast, higher plants, insect excrements
	juvabione dehydrojuvabione	paper products balsam fir
	**3a:** JH-III R1 Me R2 Me R3 Me **3b:** JH-II Me Me Et **3c:** JH-I Me Et Et **3d:** JH-O Et Et Et	*Cecropia* silkworm, various insects
	sesamin	sesame oil
	echinolone	American coneflower
	juvocimene	sweet basil

Figure 3. Some JH-activity compounds isolated from insects and plants (Taken from Slàma, 1985).

Due to the increased synthetic work and also due to potential perspectives for the use of JH-analogues (juvenoids) in insect control, the bioassays for JH have been improved, simplified and routinized. This has created favourable conditions for further testing of JH activity in plant extracts. Extensive screenings of plants for JH activity have been made by

Jacobson and his co-workers (for ref. see Slàma, 1985). Most of the assays for JH activity are based on an inhibitory action of JH on metamorphosis. This action is manifested by formation of giant supernumerary larval instars or by the appearance of intermediate forms, which contain mosaic distribution of morphological patterns of the two neighbouring developmental stages. An example of this JH action in a Hemipteran insect has been provided in Fig. 4.

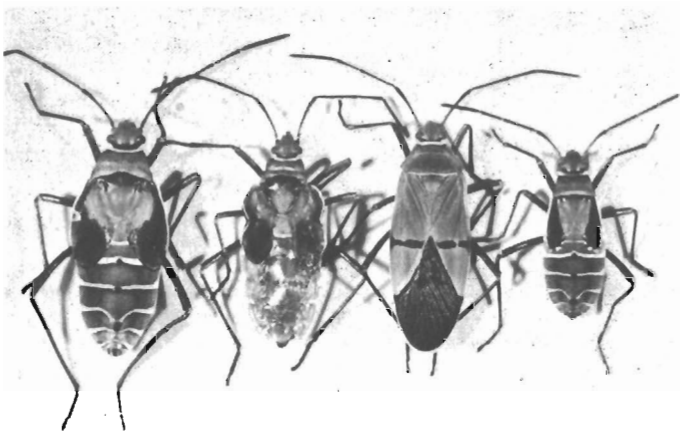


Figure 4. The effect of juvabione (5 ug applied topically to last instar larvae) on metamorphosis of **Dysdercus spp.**. From left : giant supernumerary larva, larval-adult intermediate, normal untreated adult, normal last-instar larva.

In most instances, the plant extracts have been traditionally tested for JH activity by means of topical application. It may be argued, however, that plants do not interact with their insect feeders by contact as much as they do by means of the substances present in the food. Moreover, it turns out that many synthetic, and perhaps also natural, JH-analogues including those which are little active or inactive in topical assays, may be up to thousand times more effective when administered in the diet (Slàma, 1981). This points out that plants might actually contain many indetected and ecologically important JH-active compounds, which escaped our attention due to the specialization for topical assays.

Similarly like in the other hormonomimetic studies, the common denominator for all the natural and synthetic JH mimics can be found in the determined structural and physical properties. These are related mainly to size of the molecule, exact location of the alkyl substituents and functional groups, to stereochemical orientation of the basic chains, lipophility and other physico-chemical properties (Slàma et al., 1974). According to the current pharmacokinetic theories, the above structural requirements result from specific interactions of the hormone ligands with the intracellular receptor sites. The requirements for such allosteric binding with the receptor of JH of insect represent an important factor in

the discrimination between the hormonally active and inactive molecules. The characteristic sign of JH receptors within insect epidermal cells is that they give more freedom for the binding of ligands, by contrast to the receptors of all other animal hormones, including also estrogen. Due to this, there are large structural variations tolerated among the JH mimics and there is also a record of more than 4000 of the known synthetic hormonal analogues (Sláma, 1985).

In spite of relatively simple testing procedures, some assays for JH can give occasionally quite equivocal responses. These are associated with some nonspecific side-effects, which usually lead to the false positive results. Due to this, certain plant extracts or secondary plant substances had been incorrectly classified as JH analogues without being the true JH mimics. As an example of a pseudojuvenile plant substance we may take azadirachtin. It induces developmental malformations superficially similar to JH-induced intermediates, but the latter are never true mosaics of the two epidermal patterns. In addition, azadirachtin never gives regular responses in the standardized JH assays (Sláma, unpublished).

3. The phytoecdysones

While the estrogen and JH mimics were found in plants before structural elucidation of the parent animal hormones, these hormonal mimics have been encountered in plants only after the polyhydroxylated sterolic structure of ecdysone became known (review by Karlson, 1966). Nevertheless, there is no other animal hormone that would be so closely connected with plant problematics. Ecdysone and 20-hydroxyecdysone or ecdysterone (Fig. 5), stimulate insect development from one ecdysis to the next. In this way they induce the characteristic moulting cycles. The absence of these hormones in insect body leads in immature stages to developmental arrest known as quiescence, hibernation or diapause. The bioassays for ecdysone are mainly based on the fact that the release of these hormones is regulated from some endrocrine centers located in the thoracic region. Thus, in the nondeveloping posterior body fragments or in the nondeveloping ligatured larval abdomens, ecdysone stimulates partial or complete puparíation (**Calliphora, Musca**) or pupation (Lepidoptera).

In 1966 Nakanishi and his co-workers (1966) provided the first evidence that some plants (**Podocarpus** trees) contained compounds with the chemical structure and biological activity similar to the just identified at that time insect hormone ecdysone. Other such findings immediately followed. They stimulated the efforts for standardization fo the bioassays (**Calliphora** test, **Chilo** dipping test) and facilitated large screenings for the "insect moulting hormone" activity in plants. In this work, hundreds of plant species have been extracted and tested and several dozens of ecdysone mimics - phytoecdysones - have been discovered. Quite an extensive phytochemical research in this direction, conducted mainly by the Japanese scientists (Takemoto, Hikino, Imai, Takeda, Nakanishi) revealed that phytoecdysones occurred in a number of taxonomically unrelated, lower and higher plants. References and a more detailed description of the facts pertaining to phytoecdysones can be found in reviews (Bergamasco & Horn, 1984; Rees, 1971; Sláma, 1979; Sláma et al., 1974).

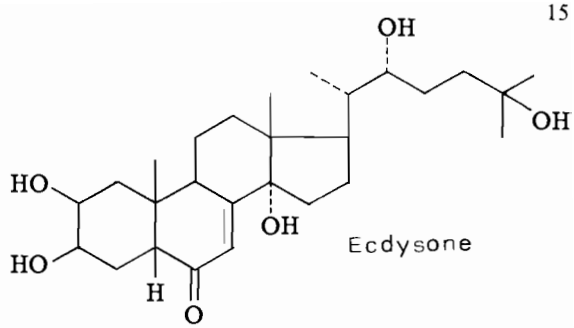
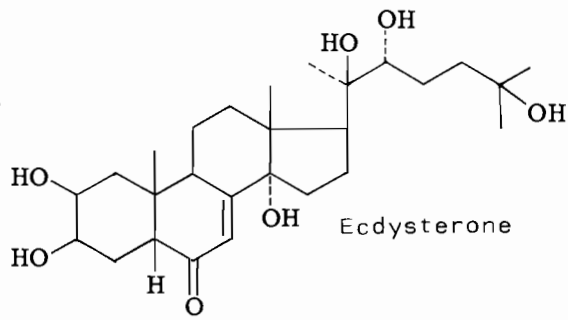


FIGURE 5.

Chemical structures of ecdysone and ecdysterone.



From the recently accumulated data on phytoecdysones (Bergamasco & Horn, 1984) we can make several conclusions: a) Ecdysone is the most widely distributed compound in plants and in insects; b) In addition to the cholestane-type compounds, plants contain also many ergostane- and stigmastane-type ones and even a few derivatives with oxidized (androstane-type) side chain; c) The hormonally active compounds from insects and plants (ecdysteroids) have identical stereoconfiguration and; d) In contrast to estrogen or JH mimics all phytoecdysones so far known have been structurally and biogenetically related to ecdysone, i.e. any nonsteroidal pharmacobiological mimic of ecdysone is still unknown.

Exact reasons why should plants contain so many phytoecdysones are not clear. certain plants, which contain a large amount of ecdysteroid (**Polypodium vulgare** ferns) appears to be almost devoid of phytophagous insects. It has been thus suggested (Slàma, 1969; Williams, 1970) that these compounds would protect some particular plants against massive attacks by phytophagous insect species. There are also more realistic views expressing doubts about real ecological value of the phytoecdysones (Beck & Reese, 1976). We have recently reinvestigated the dietaty effects of certain phytoecdysones. We are convinced that ecological importance of these compounds in insect-plant interactions cannot be ignored (Arnault & Slàma, 1986). In agreement with the results of other authors we have found that ecdysteroid concentrations from 25 to 100 ppm (0.0025 to 0.01%) would prevent larvae of phytophagous Lepidoptera from feeding on artificial diet. The common concentrations of ecdysteroids in plants range from 0.001 to 0.1% dry mass, but certain plants (**Serratula inermis**) could contain up to 2.9% (Achrem et al., 1973). Thus, these compounds may be well involved in the category of secondary plant substances that are indicated as antifeedants, feeding deterrents, repelents, phago-retardants, etc...,

which altogether forms physiological basis for a complex of factors underlying host plant selection.

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CHAPTER 1. INFLUENCE OF HOST PLANT AND ITS ALLELOCHEMICALS ON THE PHYSIOLOGY, DEVELOPMENT AND BEHAVIOUR OF PHYTOPHAGOUS INSECTS:

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SECONDARY COMPOUNDS AND INSECT HERBIVORES

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1. Introduction

Secondary plant compounds which by definition take no part in the basic metabolic processes of a plant may nevertheless fulfil significant ecological roles. A highly coloured flower pigment which is not essential for the survival of an individual plant may nevertheless be of vital importance to the survival of the species if it serves to attract pollinating insects or birds. A volatile terpene or water-soluble phenolic compound liberated by one plant may inhibit germination or growth in a competitor and there is overwhelming evidence that certain secondary compounds protect the plants that synthesise them from attack by herbivores and/or invasion by pathogens. I am concerned here with the role that secondary compounds play in the relationships which exist between plants and hervivorous insects. The subject is a complex one and one which has as yet been poorly explored. I believe, however, that its study can not only increase our basic knowledge of the way in which ecosystems have evolved but also provide us with new approaches to the control of insect pests.

Plants lack the mobility which is often an animal's first and best safeguard against predators. As a consequence, plants have evolved many different types of static defence, several of which may frequently be found in the same individual. Physical defences include thorns and prickles that deter larger herbivores, hairs on leaf surfaces that can prevent the laying of eggs by an insect and hard seed coats which can foil all but the most specialised seed eater. The scattering of seeds which makes them less readily discovered by animals can also be regarded as a behavioural trait with protective significance. It is, however, the ability of plants to synthesise compounds that are physiologically active in other organisms that provides them with one of their most important defences against predators.

2. Toxins and deterrents

The insecticidal properties of certain plants or compounds extracted from them have been known to man since earliest history. The pyrethrins from **Chrysanthemum cinerariaefolium**, rotenoids from **Derris** species, and nicotine from **Nicotiana tabacum** (the tobacco plant) provide good examples of natural products which have found widespread use in the control of insect pests. Because a secondary compound is toxic to one or more insect species is no proof however that the compounds serves to protect the plant that contains it from attack by herbivorous insects in its natural

environment. Nicotine may kill a whole range of garden pests but this in itself is no certain evidence that nicotine's insecticidal propertie can confer, or once conferred, a selective advantage, on the tobacco plant in its natural habitat. It may even be argued that such a hypothesis is untenable because the tobacco plant is not totally immune from insect attack. It provides food for a number of species including the larvae of **Manduca sexta** (the tobacco hornworm). Other apparent paradoxes include canavanine, a non-protein amino acid and close analogue of arginine, which is lethal when included at concentrations of 5% in the larval diet of the seed-beetle **Callosobruchus maculatus** (Janzen et al., 1977) but fails to protect the seeds of **Dioclea megacarpa**, which contain no less than 13% of canavanine, (Rosenthal et al., 1977) against the larvae of another seed-beetle **Caryedes brasiliensis**, and cycasin, a highly toxic carcinogen present in the leaves of the cycad **Zamia floridan**a which is nevertheless the host plant for the larvae of the hair-streak butterfly **Eumaeus atala** (Rothschild et al., 1986).

The significance of these examples lies not in the failure of the secondary compounds to protect the plants against all insect predators, but rather in what we know about the successful predators. Each of these is specifically adapted to circumvent the expected physiological effects of the principal secondary compounds present in its food plant. The larvae of **Manduca sexta** excrete nicotine (Self et al., 1964). The larvae of **Caryedes brasiliensis** possess both a highly specific arginyl-tRNA-synthetase which is able to discriminate against canavanine and thereby prevent its incorporation into protein in place of arginine (Rosenthal et al., 1976) and the ability to degrade the amino acid (Rosenthal et al., 1977). The larvae of E**umaeus atala** sequester cycasin which is eventually transferred to the pupae and aposematic adults (Rothschild et al., 1986). The presence of specific adaptations such as these in sucessful insect predators provides very strong evidence that these particular secondary compounds do have a defensive role. If they did not, it is difficult to conceive why natural selection has favoured those insects which are adapted in ways which enable them to isolate, detoxify of excrete those secondary compounds that characterise their host plants.

Secondary compounds from plants include insect antifeedants as well as toxins and it must be emphasised that toxicity and antifeedant properties are not necessarily related. The toxicity of a secondary compound can be established by means of feeding experiments and, even when toxicity can be demonstrated in more than one species, their may be marked variations in the sensitivity of different species to the same compound. Castano-spermine (1,6,7,8-tetrahydroxyoctahydroindolizine), a polyhydroxyalkaloid from **Castanospermum australe**, was found to reduce the production of adult insects by 70% when incorporated into the larval diet of **Callosobruchus maculatus** at a concentration of .005%. The same concentration of castanospermine in the larval diet of **Tribolium confusum** had no effect (Nash et al., 1986). Determining how effectively a compound acts as an antifeedant (or phagostimulant) is less easy than determining its toxicity. An insect may be given a series of choices between a control such as sugar and sugar containing increasing concentrations of a secondary compound. To

obtain statistically significant results, however, large numbers of insects are required and a plentiful supply of secondary compound must be available. The method is also very time-consuming. Schoonhaven et al. (this volume) describe research using electrophysiological methods designed to intercept and unravel the sensory code produced by the chemoreceptors of herbivorous insects in response to stimulation by either purified plant compounds or crude plant extracts (Blaney & Simmonds, 1983). The development of this technique is of particular significance as it provides a means of assessing the reaction of a specific insect, not only to individual secondary compounds but also to the complex mixture of compounds which make up a living plant. The technique also has the advantage of requiring a very small volume (200 ul) of fluid, making it possible to identify the antifeedant (or phagostimulating) properties of a plant even when little plant material is available. This is an important consideration as the availibility of plant material and the difficulties of isolating compounds in sufficient quantity for bioassay are frequently serious constraints in the study of insect/plant relationships. The technique can be used moreover to facilitate the isolation of an antifeedant (or stimulant) as the biological activity of individual fractions can be assayed during extraction and purification procedures.

Insects may be prevented from feeding by the presence of a wide variety of secondary compounds including terpenoids, flavonoids and alkaloids. The reaction of different insect species to the same compound may vary greatly, however. The larvae of **Spodoptera exempta** are completely deterred from feeding by very low concentrations of azadirachtin which occurs in **Azadirachta indica** (the neem tree). The larvae of the related species **S. littoralis** are relatively insensitive to this compound, however, and will ingest it even though it is toxic to them. In contrast, the polyhydroxyalkaloid 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine, which deters **Locusta migratoria** from feeding at concentrations as low as 0.001%, is non-toxic to the insect when force-fed in gelatin capsules (Blaney et al., 1984). It must also be appreciated that minor structural differences between secondary compounds may be accompanied by significant differences in their biological activity in insects or other organisms.

3. Attractants and phagostimulants

In their paper on kairomones, Reinbold and Tober (this volume) describe the detection of sesquiterpene alcohols and hydrocarbons in extracts of **Cajanus cajan** (the pigeon pea) which attract egg laying **Heliothis armigera** to this its host plant. The ability of a female insect to find the correct host plant on which to lay her eggs may clearly involve more than one of her senses but it is not unreasonable to suggest that the ability of an egg laying female to detect very low concentrations of volatile compounds originating from the host plant may be no less important to the survival of an insect species than the ability of the male to detect low concentrations of pheromones produced by the female.

Schoni and Stadler (this volume) have studied the effects of various chemicals present in the leaves of cabbage on the egg laying behaviour of a number of insects that are pests on that plant. Although the presence of

glucosinolates (mustard oils) is undoubtedly an important factor in stimulating egg laying in adults and/or feeding in the larvae of cabbage insects, these authors emphasise the importance of the total chemical profile of the plant to the insect. This point has also been made by van der Meijden and van Zoelen (this volume) in relation to plant selection for oviposition by **Tyria jacobaeae** (the Cinnabar moth). These authors have shown that selection of individual plants of **Senecio jacobaea** (ragwort) is related to both the concentration of water-soluble carbohydrates and protein nitrogen plants, which are richest in soluble carbohydrates, are poorest in protein nitrogen. The insects avoid plants that are either very rich or very poor in soluble carbohydrates or protein nitrogen. Commenting on their results, they state that "the absence of a positive response of oviposition on protein nitrogen at high concentrations may be due to protein nitrogen itself, to the composition of the amino acids, or to an unfavourable factor associated with a high protein nitrogen concentration. There are at least two associated factors in this animal-plant interaction: alkaloids are positively correlated with protein nitrogen while water-soluble carbohydrates (sugars) are negatively correlated".

4. Discussion

Secondary compounds in plants may act as attractants or repellants, phagostimulants or antifeedants, nutrients or toxins to phytophagous insects. When we ascribe any form of biological activity to a plant compound, we must be careful to identify the insect or insects in which the particular effect has been observed. A compound which acts as a toxin to the majority of potential predators in a plant's environment may serve as an attractant or stimulant to a specialist feeder which is not adversely affected by that compound. This apparent reversal of response on the part of the specialist feeder is probably related to the selective advantage enjoyed by an insect capable of choosing a host plant that is unavailable to most of its competitors.

In discussing the biological activity of a particular compounds we must always be aware, however, that an insect under natural conditions may be responding not merely to a given concentration of a specific compound but to the total chemical profile of the plant in which the compound occurs. This profile may vary, not only from species to species but also from individual to individual, or even from leaf to leaf and hour to hour in the same plant. The development of electrophysiological techniques capable of providing information on how an insect perceives not only single compounds but complex mixtures must therefore be welcomed as a major advance, opening as it does a new door to our clearer understanding of the ways in which insects themselves identify those plants that are suitable for purposes such as laying their eggs or eating and those that are to be avoided.

At the biochemical level, the elucidation of the ways in which plant toxins affect insects is of considerable economic importance. Knowing that a secondary compound disrupts a metabolic pathway present in insects but not in mammals or interferes with an enzyme system present in one insect but not in another could well be of significance in developing new pest

control agents.

The development of a method for evaluating the response of specific insects to compounds or mixtures of plant compounds that affect patterns of behaviour in insects is perhaps even more important, however. To have an insight into the way that a particular plant or part of a plant can attract, stimulate or repel (without necessarily poisoning) an insect provides an opportunity to advance our understanding of this fascinating interface of plant and animal biology that makes simultaneous demands on so many of the traditional scientific disciplines.

It also provides an opportunity to develop methods for the control of insect pests which do not damage an entire section of the ecosystem.

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KAIROMONES IN LEGUMES AND THEIR EFFECT ON BEHAVIOUR OF HELIOTHIS ARMIGERA

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1. Introduction

Heliothis armigera (Hübn.) is a major pest of various legumes mainly in India. The annual loss of the two major pulses, chickpea and pigeonpea, has been estimated to exceed $ 300 million per year and losses in other crops subtantially add to this total (Reed & Pawar, 1981). It is commonly considered that **Heliothis spp.** are becoming an even increasing problem due to improving and extending agricultural practices.

We are initiated a study on the chemical basis of **Heliothis armigera** resistance of chickpea (**Cicer arietinum**) and pigeonpea (**Cajanus cajan**) varieties in collaboration with ICRISAT India (Rembold & Winter, 1981). Our main interest is concentrated on such volatile compounds which, when released by the plant, strongly affect the pest insect's behaviour. Such allelochemicals were shown to be present in chickpea seeds, where they strongly attract **H. armigera** larvae (Saxena & Rembold, 1984), and in pigeonpea leaves, where they attract the pest insect for oviposition (Rembold & Tober, 1985). Here, by use of a standardized laboratory assay, the differential attraction by two pigeonpea cultivars was tested and the volatile signals, acting as kairomones, were found in the more volatile fractions of the mono- and sesquiterpenoids. This group of compounds was then further fractionated through capillary gas chromatography and their aromagrams were used for a characterization of several pigeonpea varieties with different susceptibility to **Heliothis** attack. In continuation of these studies we are now able to characterize pigeonpea varieties of different susceptibility to **H. armigera** attack by use of a bioassay, and analytically by computerizing their typical aromagrams.

2. Materials and methods

Chemicals. All chemicals were of analytical grade and were purchased from Merck (Darmstadt) or from Merck India (Bombay). Hexane (laboratory grade) was redistilled without further fractionation.

Gas chromatography, mass spectrometry. Details for these techniques have been published already (Rembold & Tober, 1985). They were identically used for this study.

Plant materials. The pigeonpea cultivars ICP-7203 (susceptible), ICP-7349 (intermediate, check), and PPE-45 (resistant to **Heliothis** attack) were grown on the pesticide free area of ICRISAT. The leaves were collected at

the plants' flowering stage. Extraction of plant materials, steam distillation, and fractionation of hexane extracts has been described elsewhere (Rembold & Tober, 1985).

Insects and oviposition test. The techniques as described elsewhere (Rembold & Tober, 1985) have been used also for this study.

3. Results and discussion

In our assay system, contact with the source of a stimulus is carefully avoided by use of a rectangular chamber with glass walls on its side and wire-nets on its two opposite ends (Fig. 1). Seedlings can thus be placed without allowing contact for the egg laying female. In most cases they layed their eggs onto the wire-net. There, the eggs were counted after a twelve hours test period. For testing plant extracts, a similar procedure was followed. In this case, a plastic disc, either containing the material to be tested or being empty, was fixed to the wire-net wall of the chamber.

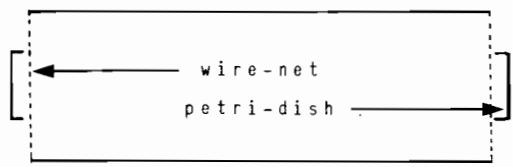

Fig. 1. Type of laboratory assay as used for the measurement of oviposition preference of **Heliothis armigera**.

Keeping in mind that oviposition behaviour is influenced by different factors like visual or contact stimuli, still a clear effect through a volatile stimulus can be demonstrated which is in accordance with the pest damage under field conditions, the less susceptible pigeonpea variety being less preferred for oviposition, and vice versa. The kairomone can be extracted with petrol ether and the soluble material, after evaporation of the solvent, stimulates oviposition on the wire-net of its side.

In the field **Heliothis** females lay their eggs near to the flowers preferentially. This is also reflected by a strong stimulatory effect of flower extract (Table 1). However, an extract from leaves is also active and the stimulus is even stronger in the steam distillate of the leaves.

Concerning the significance of these data, one has to keep in mind how complex the final decision for egg laying is, especially after only one part of the whole sequence of signals is offered to the female in the test, i.e., the olfactory one. As has been shown in our earlier study (Rembold & Tober, 1985), oviposition preference and the number of eggs layed by an individual **Heliothis** moth seem to be linked to each other in such a way that females laying less than 200 or more than 500 eggs per night are less selective in their preference behaviour than the middle group. For the present tests, all the data were used for calculation, however.

Table 1. Response of **Heliothis armigera** females, through egg laying, to a chemical stimulus contained in the hexane extracts from flowers, leaves, and the steam distillate of leaves

stimulus source	total egg counts on side of stimulus	blank	preference (%) mean	std. err.	n
Flower extract	674	516	+ 11.4	± 6.51	7
Leaf extract	993	745	+ 7.3	± 12.70	7
Steam dist. of leaves	1666	1254	+ 14.2	± 5.40	16

For reason of convenience, we have used the steam distillate for further concentration of the pigeonpea kairomone through adsorption on a silica gel colomn and stepwise elution with solvents. Although activity was eluted by hexane already (Table 2), the bulk of it was contained in the hexane/ether (1:1) fraction. Pure ether did not elute any additional material with activity in our oviposition preference test. It is clear from these data that there exists a volatile material in the pigeonpea leaves which attracts egg laying **Heliothis** females and which can be concentrated by the usual analytical techniques into a partially purified fraction. By the course of fractionation activity is not reduced, which indicates that the kairomone is composed of substances with similar characteristics if it is not a single chemical compound.

Table 2. Response of **Heliothis armigera** females, by oviposition preference, to fractions from pigeonpea leaves eluted from silica gel. Amount of material equivalent ot 1 ml of original extract.

eluent	total egg counts on the side of stimulus	blank	preference (%) mean	std. err.	n
Hexane	2338	1873	+ 10.9	± 4.22	12
50% Eth./hexane	820	624	+ 23.0	±12.10	5
Ether	925	931	+ 1.6	± 18.08	6

The material eluted by hexane/ether (1:1) from silica gel (Table 2) is, due to the fractionation pattern in electron impact mass spectrometry, composed of mono- and sesquiterpenoids mainly. They could be further fractionated by capillary column gas chromatography and, after GC-mass

spectrometry, identified in their chemical structure. The method of high resolution capillary gas chromatography was further useful for characterization of pigeonpea varieties through their aromagrams as exemplified in figure 2. In this case, the peak area of the signal most to the left in figure 2, was set equal for all the three aromagrams and by that procedure they could directly be compared with each other.

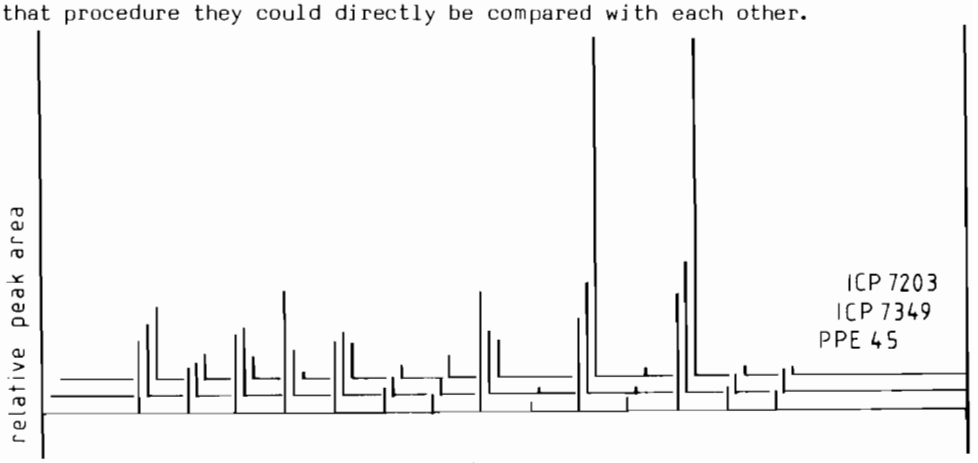

Figure 2. Aromagrams of the hexane/ether fraction from three pigeonpea varieties. The peak area of each GC signal is calculated on the basis of the first signal on the left side which is set equal for each of the three GC measurements and the signals arranged in the order of their elution from the GC column. PPE-45 is resistant, ICP-7349 of intermediate, and ICP-7203 of high susceptibility to **Heliothis** attack.

The three pigeonpea varieties are in all their GC signals qualitatively identical (with one exception of a minute signal of ICP-7203 which might have been missed). This is an important fact, at least for this hexane/ether fraction. It indicates that in the whole breeding program from which each of them has arisen, no gene has been supressed. The peaks are most prominent in ICP-7203 which is the most susceptible variety.

Vice versa, two other signals are more prominent in PPE-45, the variety which is resistant against **Heliothis** attack. None of these signals can be attributed to higher or lower susceptibility, however. Work is in progress to identify some of them and then to test them in the bioassay.

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HOST AND NON-HOST PLANT CHEMICALS INFLUENCING THE OVIPOSITION BEHAVIOUR OF SEVERAL HERBIVOROUS INSECTS

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1. Introduction

The relatively few studies based on identified and quantified plant compounds influencing the oviposition behaviour of phytophagous insects seem to indicate that polyphagous species ("generalists") and mono- and oligophagous species ("specialists") are using different chemical cues to locate and recognize their preferred host plants (Renwick, 1983). While discrimination by "generalists" is thought to rely mainly on repellent or deterrent plant compounds which occur in avoided plants (Jermy & Szentesi, 1978), host finding and acceptance by "specialists" would be mediated by specific key stimuli present in this combination only in their preferred hosts (Feeny, 1986; Städler, 1982, 1986; Visser, 1986). This hypothesis seems to be in contradiction to the conclusions derived from a comparative study of contact chemoreception of different Lepidoptera larvae by Dethier and Kuch (1971) and Dethier (1973), who found no principal difference in the chemosensory response of poly- and monophagous insects to plant saps and compounds of host and nonhost plants. Even though only contact chemoreception of larvae was investigated it shows that the hypothesis of different chemical perception of plants by insects with different host plant selection strategies remains to be investigated in much more detail.

We are studying this question by comparing the oviposition behaviour and sensory physiology of adult insects with different host specificities and by analysing the behaviourally active compounds of one plant, cabbage. This is the first contribution reporting results of the comparison of responses of the following insects to raw cabbage extracts and its fractions:

- the cabbage root fly, **Delia radicum**, a "cabbage specialist"
- the cabbage butterfly, **Pieris** (**Artogeia**) **rapae**, a "cabbage specialist"
- the cabbage looper, **Trichoplusia ni**, a "generalist" ovipositing on cabbage
- the carrot fly, **Psila rosae**, a "specialist" on Umbelliferae, never ovipositing on cabbage.

2. Materials and methods

2.1 Rearing conditions

The cabbage root fly **Delia radicum** and the carrot fly **Psila rosae** were reared in the laboratory at the EFA Wädenswil according to standard methods (Finch & Coaker, 1969; Städler, 1977). The small cabbage butterfly **Pieris rapae** and the cabbage looper **Trichoplusia ni** were reared and assayed at the

BTI Ithaca (Renwick & Radke, 1981, 1983).

2.2 Extracts and fractions

For the preparation of the extracts used in the oviposition bioassays one single race of cabbage, **Brassica oleracea** var. Golden Acre, was used. The extraction and fractionation procedure was as follows: Freshly collected cabbage leaves were added to boiling ethanol to prevent enzymatic breakdown, and after cooling homogenized with a blender. The ethanolic raw extract was filtered and evaporated to dryness. The residue was first extracted with hexane and then with water. The water fraction was extracted with butanol producing a butanol and a post butanol water fraction. The butanol fraction was separated by a HPLC C18 reversed phase column with a water-acetonitrile gradient into the three fractions A, B and C (Fig. 1).

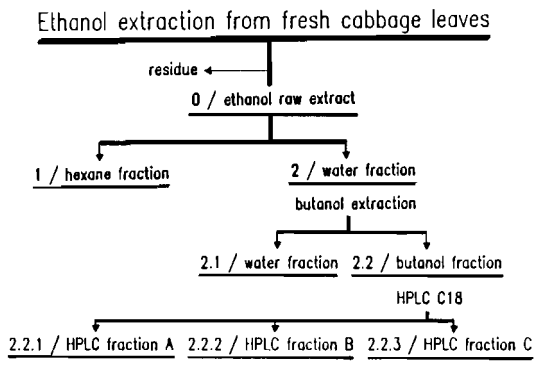


Figure 1. Fractionation diagram

2.3 Bioassays

Depending on the oviposition behaviour of each species a different specific method was applied to test the influence of the fractions of the raw extract on oviposition. In the case of the cabbage root fly the test solutions were applied with a chromatography sprayer onto the whole surface of an artificial leaf covered with a thin layer of paraffin wax (m.p. 44-45°C) (Schöni & Städler, in prep.). For each replicate three models were sprayed with 1 ml of a test fraction with a concentration of 0.1 gram leaf equivalent (gle) each and three identical models with 1 ml of the pure solvent (controls). Test and control substrates were alternated in a circle on the bottom of a test cage. Each test cage contained about 50-100 mature male and female flies. The eggs were counted daily and for each pair test/control a discrimination index was calculated as the difference of eggs laid on each test and corresponding control substrate in percent of the total of eggs laid on both subtrates. Statistical analysis of the discrimination indices was performed by the nonparametric Friedman sign rank test.

For the cabbage looper, the test and control solutions were sprayed onto the surface of intact young cabbage plants (Golden Acre) as described by Renwick and Radke (1981). The plants for test and control were of the

same age (about 8 weeks). For each replicate 15 ml with 0.2 gle/ml was sprayed evenly on the whole surface of the plant.

For the small cabbage butterfly the solutions were applied on green index cards (12.7*7.6 cm) mounted on a wooden stick (Renwick & Radke, 1983).

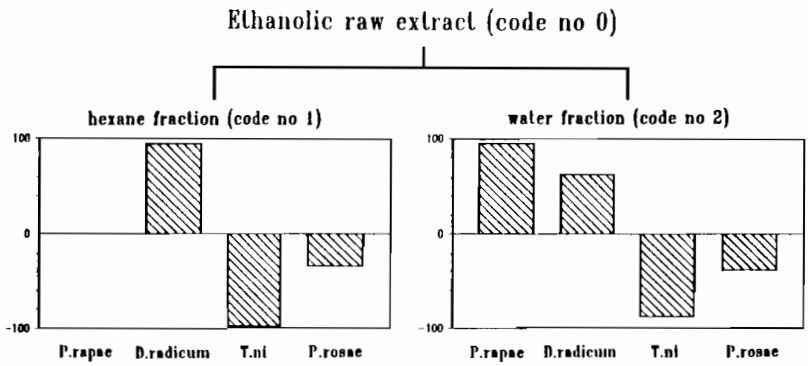
For the test of the carrot fly 1 ml solutuions with 5 gle were sprayed onto the whole surface of freshly collected carrot leaf. For each pair test/control the leaves were taken from the same plant and of about the same size. The leaves were mounted on moist artificial substrates as described by Städler (1977). Six pairs of test/control were alternated in a circle on the bottom of a cage (70*70*70 cm) with about 100 flies of both sexes. For the statistical analysis the Wilcoxon sign rank test for paired observations was used.

3. Results and discussion

The results of a first series of bioassays with the hexane and water fraction of the raw cabbage extract are shown in figure 2. A comparison of the response of the two "specialist" cabbage species **Pieris** (**Artogeia**) **rapae** and **Delia radicum** shows that the two fractions are not equally important for oviposition stimulation in both species. For P. **rapae** almost all stimulatory factors were polar compounds eluted in the water fraction. In contrast, for **D. radicum** polar as well as nonpolar compounds had a stimulatory effect on oviposition.

The same fractions which were neutral or stimulatory for the "specialists" were highly repellent and/or deterrent for the generalist **T. ni**. The compounds extracted from the host plant leaves of **T. ni** must be sufficiently potent deterrents or repellents to mask possible stimulants. This is surprising but can be explained by the fact that the cabbage looper is repelled or deterred from egg-laying on a plant damaged by larvae feeding on it (Renwick & Radke, 1981).

If the response of the generalist **T. ni** is compared with that of the "specialist" **Psila rosae** it is evident that the inhibitory effect of both fractions is much more pronounced in the case of **T. ni**. This is again surprising in view of the act that **T. ni** oviposits on cabbage whereas **P. rosae** does not. Furthermore for **P. rosae** a higher concentration of the fractions were necessary (5 gle per leaf) to obtain significant inhibitory effects. This seems to support the idea that polyphagous insects such as **T. ni** are very sensitive to deterrents and repellents (Jermy & Szentesi, 1978; Renwick, 1983). Comparative EAG recordings from the antennae **T. ni**, P. **rapae** and **Papilio polyxenes** did indeed show that **T. ni** is very sensitive to repellent extracts and compounds (Renwick & Radke in prep., Städler et al., in prep.). At this point caution is needed to prevent premature conclusions. Since for both **T. ni** and **P. rosae** their respective host plants were treated with the extracts, inhibitory effects may not only be caused by repellents and/or deterrents but also by masking of stimulants on the leaf surface. For the carrot fly the presence of stimulants on the leaf surface have been proven (Städler & Buser, 1984) and thus masking could indeed explain the observed inhibitory effects of applied cabbage extracts. Experiments with isolated compounds are needed to resolve this question.



the two initial fractions of the raw ethanol extract :
Delia radicum (D. radicum), N=6, 1 ml of 0.1 gle/ml solution/artificial leaf (0.1 gle/artificial leaf).
Pieris rapae (P. rapae), N=6, 1 ml of 5.0 gle/ml solution/artificial leaf (5.0 gle/artificial leaf). No eggs with hexane fraction.
Trichoplusia ni (T. ni), N=8, 15 ml of 0.2 gle/ml solution/plant (= 3 gle/plant).
Psila rosae (P. rosae), N=12-18, 1 ml of 5.0 gle/ml solution/leaf (= 5 gle/leaf).

a) **Delia radicum**

b) **Psila rosae**

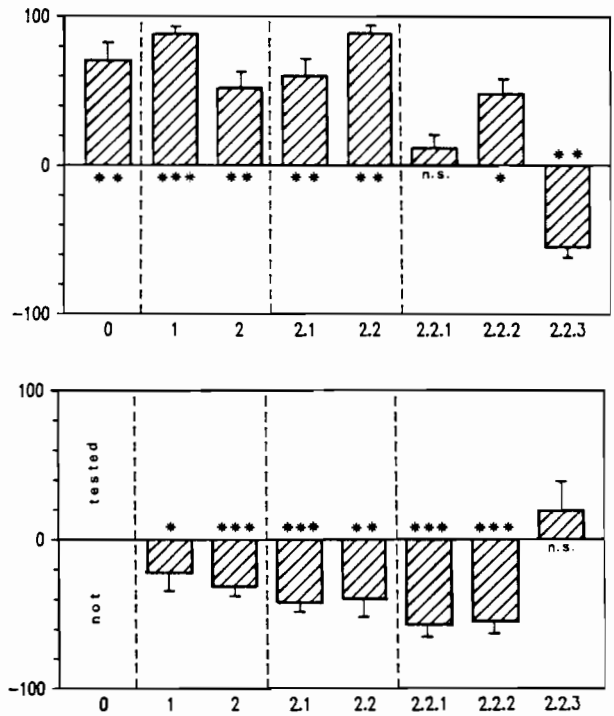


Figure 3a, b. Mean discrimination indices and standard errors (S.E.) of bioassays with several fractions of the raw cabbage leaf extract.

Finally, the comparison of results (Fig. 2, 3) of **D. radicum** and **P. rosae** leads to the impression that the negative response of the non-cabbage insect **P. rosae** to one fraction is correlated with a positive response of the cabbage insect **D. radicum** to the same fraction. However, the bioassay results of the further fractionation of the water extract (2.1) show (Fig. 3a, b) that the HPLC fraction A (2.2.1) which proved to be highly repellent and/or deterrent for **P. rosae** was weakly stimulatory (not significant) for **D. radicum**. On the other hand the HPLC fraction (2.2.3.) inhibited (significantly) the oviposition of the cabbage flies on the treated test leaves. For **P. rosae** the same fraction was not inhibitory but seemed to be even slightly stimulatory (not significant, $p > 0.1$).

4. Conclusions

The present results with fractions of leaf extracts seem to support the following conclusions:

1) Several different compounds (with different chemical characteristics) and not only glucosinolates do influence the oviposition behaviour of all investigated species
2) Each species must have a specific chemosensory perception of the cabbage leaf extracts which is translated into a stimulation or inhibition of oviposition.
3) Species with similar host plant specificities are not stimulated by the same fractions (compounds) showing that comparative studies are important.
4) The active leaf compounds have to be isolated and identified in order to study their combined effect (interaction) on the oviposition behaviour and the chemoreceptors of the different insects.

Abstract

The influence of compounds extracted from cabbage leaves on the oviposition behaviour of the two "specialist" cabbage insects **Delia radicum** and **Pieris** (**Artogeia**) **rapae**, the "generalist" cabbage insect **Trichoplusia ni** and the carrot fly **Psila rosae** was tested in laboratory bioassays using natural and artificial egg laying substrates. Raw extracts releasing or inhibiting oviposition were separated by analytical techniques into purified fractions to isolate and identify the active chemical compounds. A comparison of the data shows that the decision to accept or reject a plant as a suitable host is not uniquely based on some few key stimuli, e.g. the glucosinolates, but rather on a large variety of stimulatory and inhibitory plant chemicals acting together. In addition, the "chemical image" of the cabbage leaf (extracts) appears to be specific for each species.

Acknowledgements

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PLANT GROWTH HORMONES : THEIR PHYSIOLOGICAL EFFECTS ON A RANGELAND GRASSHOPPER (AULOCARA ELLIOTTI)

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1. Introduction

Although grasshoppers and locusts periodically ravage vegetation in their habitats, these insects and their host plants remain extant. Thus, both the insects and the plants that they feed on are adapted, not only to the physical factors in their environments, but also to each other. Our understanding of the adaptive mechanism(s) that permit these insect-plant relationships is still incomplete. Studies to determine what factors underlie the enigmatic fluctuations of populations of **A. elliotti** in Montana revealed that winter moisture and spring/summer growing temperatures of its host grass (western wheatgrass, **Agropyron smithii**), independent of the rearing temperature of the insect, can significantly affect the laying of viable eggs (Visscher et al., 1979). Because plant growth hormones (PGHs) change in kind and concentration with variations in temperature, moisture, and daylength, as well as with aging in the plant (Leopold & Kriedmann, 1975), it seemed possible that changing concentrations of these substances in host plants might be related to the physiological and reproductive changes that result in grasshopper/locust outbreaks.

An initial experiment to test this hypothesis demonstrated that gibberellic acid (GA_3) and abscisic acid (ABA) added to leaves of **A. smithii** as they were fed on by pairs of **A. elliotti** could significantly inhibit their production of viable eggs (Visscher, 1980). GA_3, however, was most effective at a concentration 10-fold higher than the most effective dose of ABA. Subsequently when indoleacetic acid (IAA), GA_3, and kinetin (a synthetic cytokinin) were fed with this grass at other concentrations to adult **A. elliotti**, highly significant increases in their reproductive success and longevity were observed (Visscher, 1982). Because PGHs regulate plant metabolism, it was uncertain whether these results reflected direct effects on the physiology of the insects or indirect effects brought about by an altered nutrient status of the host plants. To resolve this uncertainty experiments were undertaken in which nymphs and adults of **A. elliotti** were fed PGHs at three concentrations in a defined diet devoid of unrefined plant materials and several physiological and morphological traits of these variously treated insects were compared.

2. Materials and methods

Newly hatched nymphs of **A. elliotti** were reared three males and three females/cage using published methods (Visscher, 1971). These nymphs were

fed either a defined diet (modified from Dadd, 1960) devoid of unrefined plant materials or field-grown grass. The latter was harvested on June 15th from a clone of **A. smithii** grown in a field near Bozeman, freeze-dried, and ground into a powder. These control diets were formed into uniform pellets and fed to groups of about 30 nymphs. PGH treated groups of 20 nymphs were fed equal amounts of defined diet with 2, 10, or 50 ppm of GA_3, IAA, zeatin (a natural cytokinin), or ABA added. Other groups of 20 nymphs were fed defined diet containing 20% by volume wheatgrass clone grown at the field site, or grown at warm (24-29°C) or cool (18-24°C) temperatures in controlled environment chambers. Cages were maintained in a greenhouse insectary with diurnally fluctuating temperatures and natural daylengths supplemented with light from halide lamps (12 hr light, 12 hr dark).

A second series of nymphs was reared on fresh western wheatgrass from the field-grown clone from hatching to adult ecdysis, then isolated as single pairs and maintained thereafter until death on the defined diet, with or without PGHs. In this experiment kinetin was substituted for zeatin and PGH concentrations were raised to 5, 25 and 50 ppm. Adult pairs were reared under natural daylengths at 30°C day: 25°C night temperatures. Egg pods were collected daily and incubated at 25°C for 30 days, then fixed in Bouin's solution and stored at 70% ethanol. Viability was assessed microscopically according to criteria of Van Horn (1966a). Ten physiological or morphological characteristics of nymphs or adults were compared between the various treatment groups. The results of both experiments were analyzed by analysis of variance.

3. Results and discussion

Groups fed different dietary regimens showed highly significant differences in the following characteristics: nymphal rate of development, nymphal survival to the adult stage, time from the adult ecdysis to laying of the first eggs, elytron length, elytron/femur ratio, femur/head width ratio, body weight of newly molted adults, fecundity, egg viability, and adult longevity. Data comparing effects of feeding exogenous GA_3 in the defined diet versus a diet containing no exogenous PGHs are presented below. Because of spatial limitations the results of studies of other hormones will be summarized here and detailed results will be presented elsewhere.

The data presented in Table 1 demonstrate that nymphal survival is reduced by GA_3 fed at the low concentration, unaffected by GA_3 at the 10 ppm concentration, and reduced by GA_3 fed at the highest concentration. Nymphal rate of development was significantly increased by the addition of GA_3 to the defined diet at all three concentrations.

GA_3 is apparently required for reproduction of **A. elliotti**. Adults fed GA_3 in the defined diet laid fewer eggs than the controls fed freeze-dried wheatgrass or untreated defined diet (Table II). However, only females fed GA_3 at the highest concentration laid any viable eggs, and although the number of fertile females was reduced from those in the control groups, the mean number of viable eggs laid by females fed GA_3 at the 50 ppm dose was larger than that in other groups. Moreover, the days to laying the first egg pod after the adult ecdysis was significantly shortened in females that

were fed the highest concentration of GA_3 in the defined diet.

The addition of other PGHs to the defined diet affected nymphal development and survival. The addition of 20% freeze-dried wheatgrass to the defined diet resulted in the highest percentage of nymphal survival. Nymphal survival was increased by ABA at lower doses compared to that of nymphs fed untreated defined diet. Nymphal development was delayed by all concentrations of zeatin, whereas GA_3, IAA, and ABA at all concentrations shortened the time to adult ecdysis. The time between adult ecdysis and laying of the first egg pod was shortened by the addition of IAA to an even greater extent than by GA_3.

Table I. Effects of exogenous gibberellic acid fed in a defined diet to nymphal Aulocara elliotti.

Dietary Regimen	Initial Number	Percent Survival	Days to the Adult Ecdysis	P-value
Def. Diet Untreated	31	35.5	40.1 ± 3.9	---
Def. Diet + GA_3 2ppm	29	27.6	33.0 ± 4.8	.04
Def. Diet + GA_3 10ppm	31	38.7	35.5 ± 4.9	.03
Def. Diet + GA_3 50ppm	30	16.7	34.6 ± 2.4	.002

Table II. Effects of exogenous gibberellic acid fed in a defined diet to adult Aulocara elliotti.

Dietary Regimen	Initial Number	Fertile Females	Mean Days to First Eggs	Mean Eggs /Female	Mean No. Viable Eggs
Def. Diet Untreated	30	11	21.2 ± 4.38	26.2	.55
Dried wheatgrass	30	21	23.9 ± 4.91	15.9	.55
Def. Diet + GA_3 5ppm	20	3	29.0 ± 8.21	2.3	0.00
Def. Diet + GA_3 25 ppm	20	5	26.3 ±20.00	8.7	0.00
Def. Diet + GA_3 50ppm	20	4	17.5 + 4.04	6.3	4.50

Unexpectedly, several morphological characteristics were affected by exogenous PGHs. The elytron lengths of adults fed dried wheatgrass as nymphs were longest, but the addition of ABA at the highest concentration resulted in significantly increased tegmen lengths compared to those of adults fed other dietary treatments. Elytron/femur ratios were decreased by the addition of zeatin to defined diet, whereas ABA at 50 ppm increased that ratio compared to that observed in the group fed untreated diet. Zeatin, on the other hand, fed at the lowest dose resulted in significantly increased femur/head ratio in adults fed that hormone as nymphs.

Results of these experiments demonstrate that developmental rates, morphology, and reproductive performance of **A. elliotti** are related to the kinds and concentrations of PGHs added to a defined diet devoid of unrefined plant materials. They are the first to show direct effects in these herbivorous insects. It seems likely that changes in the kinds and concentrations of ingested PGHs - appearing as host plants respond to changing temperatures, moisture, and daylength conditions - play an important role in correlating the physiology of **A. elliotti** with seasonal as well as unseasonal changes in the physiological condition of its host plant **A. smithii**. Correlation of insect growth and reproduction to the condition of the host plant through PGHs could ensure that insects feeding on plants under drought and temperature stress would adjust their performance in ways that are adaptive to the long-term survival of both species. That primary plant compounds, as well as secondary plant metabolites, have direct effects on the metabolism of insects is not surprising in view of our present understanding of the evolutionary derivation fo the intermediary metabolism of animals from plant-like ancestors. These findings suggest more complexity in the biochemical relationships of herbivores with their host plants than was previously recognized.

Abstracts

Nymphal and adult **A. elliotti** fed three concentrations of each of four plant growth hormones (gibberellic acid, indoleacetic acid, cytokinin, or abscisic acid) in a defined diet devoid of unrefined plant materials exhibited significant differences in ten characteristics: nymphal survival, nymphal rate of development, elytron length, elytron/femur ratio, femur/head width ratio, adult body weight, fecundity, egg viability, time from adult ecdysis to laying of the first eggs, and adult longevity. This observation of direct effects of plant growth hormones on the physiological and morphological characteristics of grasshoppers supports the hypothesis that changing kinds and concentrations of plant growth hormones resulting from seasonal or unseasonal changes in physical factors on the plant may play a role in correlating the insect's growth and reproduction with changes in the physiological condition of its host plant. Such a biochemical adaptive mechanism could help to explain why grasshoppers and locusts in plague numbers have not destroyed their host plant species.

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Labeyrie, V., Fabres, G., Lachaise, D. (eds) Insects – Plants

THE PHYSIOLOGICAL EFFECTS OF AZADIRACHTIN IN THE LOCUST, LOCUSTA MIGRATORIA

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1. Introduction

Azadirachtin, a limonoid from the neem tree **Azadirachta indica**, is a most effective antifeedant and toxicant against many insect pests (see Warthen, 1979; Schmutterer & Ascher, 1985 for examples). The toxicology of azadirachtin is not well understood. Its insect growth regulatory effects have led to the suggestion that azadirachtin might act directly on the neuroendocrine system by modifying haemolymph ecdysteroid titres (Redfern et al., 1982; Sieber & Rembold, 1983; Schluter et al., 1985). However it is not known whether these effects are direct or indirect. A direct effect of azadirachtin which has recently been demonstrated is its inhibitory action on gut motility in **Locusta** (Mordue (Luntz) et al., 1985). Such lesions may provide important clues as to its mode of action. A healthy gut is required for feeding, the digestion of food for growth, the initiation of a moult after the attainment of the 'critical body mass' and successful ecdysis by the swallowing of air to split and shed the old cuticle. Perhaps some of the insect growth regulatory (IGR) effects of azadirachtin can be explained by its action on the gut without the need to postulate a direct action on ecdysteroid metabolism itself.

2. Results

IGR effects in the locust. The effects of azadirachtin on **Locusta** are highly predictable and are seen as a variety of effects related to the requirement to moult. Treatment with increasing doses of azadirachtin in the Vth instar results in (i) adults with curled wing tips and decreased longevity, (ii) Vth instar nymphs which die during ecdysis, (iii) nymphs which die just before the moult after a normal instar length, (iv) insects with a greatly extended instar and (v) insects which die soon after treatment. The LD_{50}, taking all these parameters into account, is 2 ug/g body weight. Acute toxicity (within 24 h of injection) occurs at 40 times that concentration with an LD_{50} of 80 ug/g body weight (Mordue (Luntz) et al., 1985).

The differences in the timing of mortality following different doses of azadirachtin are closely linked with slower growth of the insect and lack of feeding (Mordue (Luntz) et al., 1985). As the dose is increased food intake is reduced, growth is slower and moulting is inhibited. The moult inhibiting effects of azadirachtin are not solely due to starvation (Sieber & Rembold, 1983) however, the explanation for both the lack of feeding and moult inhibition may well lie in the effect of azadirachtin on

the gut (Mordue (Luntz) et al., 1985).

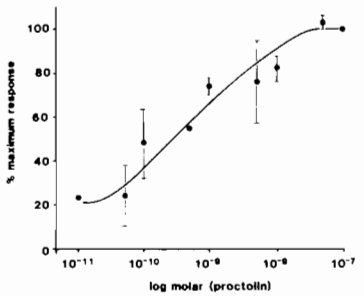

Fig. 1. Dose response of **Locusta** hindgut to proctolin in vitro (see Starratt & Steele, 1980 for method). Contractile tension is expressed as a percentage of the peak tension achieved with 10^{-7} M proctolin. The vertical lines represent the limits of SE of the mean. Each point is the mean of between 2 and 6 experiments.

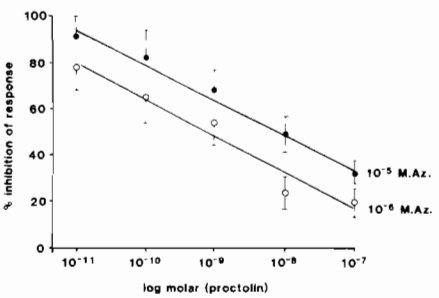

Fig. 2. The inhibition of proctolin stimulated **Locusta** hindgut by azadirachtin. Contractile tension is expressed as a percentage inhibition of the peak tension achieved at each concentration of proctolin. Azadirachtin was added 2 min prior to proctolin stimulation. The vertical lines represent the limits of SE of the mean. Each point is the mean of between 4 and 11 experiments.

Effect on the gut. It is clear from our earlier work that azadirachtin significantly reduces the basal gut contraction rate of **Locusta** gut in a dose-dependent manner (Mordue (Luntz) et al., 1985). Since then work has progressed to quantify the inhibition of azadirachtin on proctolin stimulated locust hindguts. The main effect of proctolin, an excitatory neuromuscular transmitter and neuromodulator of insect viscera, is seen on the muscle tonus as a slow graded contraction of the longitudinal muscles followed by several rapid contractions. The locust hindgut response to increasing concentrations of proctolin shows the classic dose/response effect (Fig. 1). These proctolin induced contractions can be blocked in a dose dependent fashion by azadirachtin (Fig. 2).

The inhibition of gut contraction by azadirachtin reduces the passage of food through the gut (Mordue (Luntz) et al., 1985). This results in permanently semi-full gut and a suppressed level of feeding, due to the fact that feeding is not initiated in the locust until the gut is relatively empty (Bernays & Chapman, 1973; Simpson, 1983). This effect is distinct from the feeding deterrency caused by the detection of azadirachtin by mouthpart chemoreceptors (Haskell & Mordue (Luntz), 1969; Blaney & Winstanley, 1980) and is also observed in **Pieris** (Schoonhoven & van Loon, pers. comm.; Mordue & Evans, unpub. obs.), **Manduca** (Reynolds, in press) and **Spodoptera** larvae (Redfern et al., 1980). The reduced level of feeding in azadirachtin treated insects is an important phenomenon which is often neglected and ignored in other work on the toxic action of azadirachtin, particularly because of its relevance to the attainment of a critical body mass prior to moult initiation.

Effect on Ecdysone levels. Collaborative work with Hoffmann and Charlet to measure blood ecdysteroid levels (Mordue (Luntz) et al., 1986) has shown that by treating insects with azadirachtin at the end of the instar but before the 'critical mass' is reached and before the start of the major peak, ecdysteroid release is entirely prevented (Fig. 3a). In this instance the treated insects did not reach the 'critical mass' at which ecdysteroid release would have occurred (Mordue (Luntz) et al., 1986). Similarly, in **Manduca sexta** in some instances a supernumerary larval moult is initiated after neem extract treatment (Hassler, 1985). In this instance the size monitoring mechanism of the insect which would normally have switched off the corpora allata in the last larval instar has not functioned properly.

Once the 'critical mass' and developmental competence to moult has been reached the insect initiates a sequence of events which triggers the secretion of prothoracicotropic hormone (PTTH) from the brain with the eventual release of ecdysone. The 'trigger' stimulus is related to feeding and may be due to stretching of the abdomen, as in **Rhodnius** or **Manduca**, or of the gut, as in **Oncopeltus** (Nijhout, 1981). The trigger for moulting in **Locusta** is not certain but if a maintained stretching effect of the gut is required, then azadirachtin poisoning of the gut with its reduced food intake, may well prevent any release of PTTH and hence also ecdysone release.

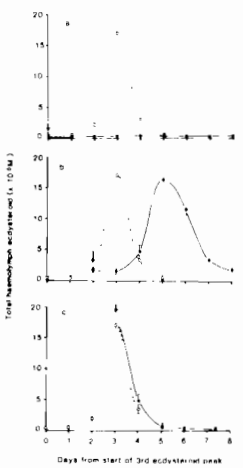

Fig. 3. Total haemolymph ecdysteroid (x 10^{-6} M) of female 5th instar nymphs of **Locusta migratoria** treated with 7.5 ug azadirachtin in 5 ul 2.0% ethanol. Controls were injected with an equivalent volume of the ethanol solution only. (a) Insects injected prior to ecdysteroid peak, death occurred before the moult. N=6. (b) Insects injected at the start of the ecdysteroid peak, death occurred before the moult. N=7. (c) Insects injected at the ecdysteroid peak, death occurred during the moult. N=9. o— — —o Controls, N=8. •——• Azadirachtin-treated. ↓ Time of azadirachtin injection.

Effect on the moult.

(a) Release of eclosion hormone. Ecdysis is under the control of eclosion hormone (EH), which is released following the rapid decline of ecdysteroid titre at the end of the instar. Release of EH triggers various stereotyped behaviours which cause ecdysis (see Truman, 1985). Azadirachtin treated insects which have reached their critical mass either before or after treatment may still die before the moult. That such insects have grown but have failed to moult suggests that azadirachtin has affected ecdysteroid levels to prevent EH release. Again, this appears to be so. Azadirachtin treatment at the start of the third ecdysteroid peak delays the peak and

slows down its subsequent decline (Fig. 3b), with the concomitant blockage in moult initiation.

Preliminary experiments with Hoffmann and Charlet, using ^{3}H ecdysteroids have been carried out to establish effects of azadirachtin on the metabolism of ecdysteroids. There are no significant differences in the metabolic fate of the injected tracers between normal and azadirachtin treated insects. Clearly azadirachtin is exerting its effect in a more indirect way, perhaps by affecting the release of PTTH.

(b) Ecdysis. Once EH has been released ecdysis occurs with the initiation of the CNS motor programme controlling the complex movements necessary for the insect to extricate itself from the old cuticle. An important event in this process is the swallowing of air by the insect to increase its volume, to split and shed the old cuticle and expand the new. One of the most common moult abnormalities seen in azadirachtin treated insects is death during ecdysis with the insect unable to extricate itself from the old cuticle. Such insects have been shown to have normal blood ecdysteroid levels (Fig. 3c), hence EH is released and the moult initiated. However these insects are unable to comlete the moult due to an inability to increase the volume of air within the body to the level required for successful moulting (Fig. 4). It is interesting to speculate whether gut expansion here has been significantly impaired by the azadirachtin treatment, in the same way that food intake is affected during the instar.

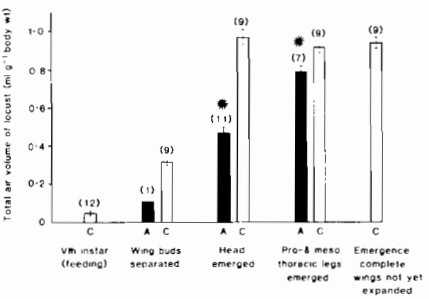

Fig. 4. Changes in the quantity of air (+ SEM) contained within the whole insect during the course of the imaginal ecdysis of male and female **Locusta migratoria** injected with 7.5 ug azadirachtin in 5 ul 2.0% ethanol 1-2 days prior to the moult. Controls were injected with 5 ul 2.0% ethanol solution only. Volumes of air were collected from azadirachtin-treated insects when they had reached each stage in the moult and could proceed no further. Figures in parentheses refer to numbers of insects/group. A=azadirachtin-treated. C=control. *, $P < 0.005$.

The diverse effects of azadirachtin upon growth and moulting are beginning to be broken down into their basic components. Azadirachtin has a direct effect on peptide neuromodulation of the gut; it may also affect release of PTTH and EH. It is interesting to speculate whether azadirachtin exerts its effects specifically on peptidergic systems or whether it has a more general effect on cellular mechanisms by affecting, for example, ion fluxes.

Abstract. Azadirachtin injection early in the Vth instart produces dose-dependent developmental aberrations: malformed adults of reduced longevity

result at low doses, death occurs during or prior to the moult at higher doses. Growth rate and food intake are reduced. In insects treated at the end of the instar, the particular effect seen depends upon the timing of injection, relative to the major haemolymph ecdysteroid peak. Azadirachtin has an inhibitory effect on proctolin stimulated contractions of the hindgut. The significance of azadirachtin treatment upon attainment of a 'critical mass', the release of eclosion hormone and successful ecdysis is discussed.

Acknowledgements

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HYDROXAMIC ACIDS FROM GRAMINEAE: THEIR ROLE IN APHID RESISTANCE AND THEIR MODE OF ACTION

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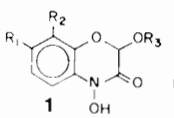
Hydroxamic acids (Hx, 1) isolated from extracts of cereals such as maize, wheat and rye (Willard & Penner, 1976) have been suggested as resistance factors against several organisms. In this paper we summarize evidence showing that Hx are involved in cereal resistance to aphids and describe biochemical and chemical bases for this biological activity.



1

R_1 = H or CH_3O
R_2 = H or CH_3O
R_3 = H or Glucosyl
DIMBOA : $R_1 = CH_3O$, $R_2 = R_3 = H$

Role of hydroxamic acids in host plant resistance. Different wheat, maize and rye cultivars showed varying Hx levels up to 1.2 g/kg fr. wt. Inverse relationships were obtained between these levels and growth rates of populations of the aphids R**hopalosiphum maidis** (Long et al., 1977), **Metopolophium dirhodum** (Argandona et al., 1980), **Schizaphis graminum** (Corcuera et al., 1982) and **Sitobion avenae** (Bohidar et al., 1986). Comparisons made were intraspecific or interspecific with respect to the plant.

Hx content varied with plant age, showing a steep increase a few days after germination and a subsequent slow decrease. Inverse correlations were found between Hx in wheat and rye plants of different ages and growth rate of populations of **M. dirhodum** (Argandona et al., 1980).

Hx content was higher in younger than in older tissues. Young wheat leaves were more resistant than old leaves towards **S. graminum** (Argandona et al., 1981).

DIMBOA, the main hydroxamic acid in wheat and maize extracts, decreased survival and reproduction rate of aphids fed with holidic diets, at concentrations comparable to those found in the plant (Corcuera et al., 1982).

These facts support the idea that hydroxamic acids constitute a chemical defense of cereals against aphids.

Additionally, hydroxamic acids have been claimed as cereal resistance factors towards fungal diseases such as **Puccinia graminis** (ElNaghy & Shaw, 1966) and **Diplodia zeae** (BeMiller & Pappelis, 1965) and towards the insect **Ostrinia nubilalis** (Robinson et al., 1978).

Biochemical bases of DIMBOA toxicity. Hydroxamic acids are toxic towards an ample range of organisms that include bacteria (Corcuera et al., 1978; Lacy

et al., 1979) fungi and insects. A biochemical basis for such widespread toxicity among aerobic organisms was sought for by examining the effects of DIMBOA on energy-linked reactions in submitochondrial particles from bovine heart (Niemeyer et al., 1986).

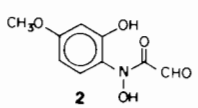
Electron transport from NADH, succinate and ascorbate + N,N,N,N-tetramethylphenylenediamine (TMPD) both in coupled and in uncoupled particles was reversibly inhibited by DIMBOA (I_{50} = 11 mM). This effect may be located at complex IV of the respiratory chain.

DIMBOA also inhibited reversibly the ATPase complex, as reflected by inhibitions of ATP synthesis, P_i-ATP exchange reaction and ATPase activity (I_{50} = 4, 2 and 6 mM, respectively).

More importantly, incubation of submitochondrial particles with DIMBOA resulted in an irreversible inhibition of electron transport when either NADH or succinate were used as substrates (loss of 50% activity was obtained with 4 mM DIMBOA in 40 min). Furthermore, in DIMBOA-treated particles cytochromes **b**, **c** and **a** were not reduced by succinate whereas cytochromes c and a were reduced by ascorbate + TMPD. Hence, this irreversible inhibition by DIMBOA may be located at complex III of the respiratory chain.

This latter effect would produce an inactivation of ATP synthesis leading to increased ineffectiveness of the energy metabolism of aerobic organisms and could be responsible for the toxicity of DIMBOA.

Chemical bases for enzymatic inhibitions by DIMBOA. DIMBOA and the open-chain analogue 2 in equilibrium with it (Copaja et al., 1986) possess several centers potentially reactive towards nucleophiles. These nucleophiles may be residues from aminoacids such as cysteine (SH groups) or lysine (NH_2 groups) related to the activity of enzymes. The reaction of DIMBOA with thiols and amines was studies in further detail.



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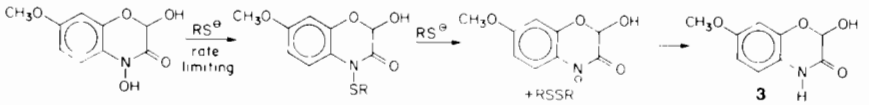
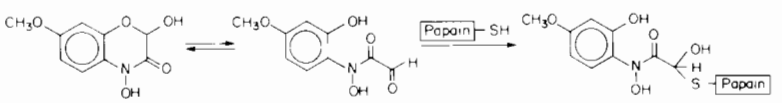
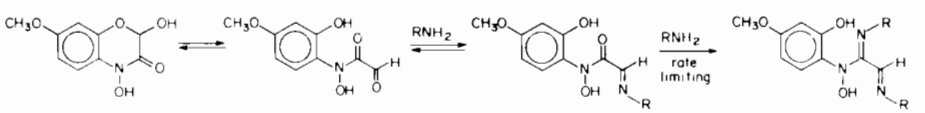
Reaction of DIMBOA with thiols. The main product from the reaction of DIMBOA with thiols was the reduction product, lactam **3**. Other products were hemithioacetals and reduced hemithioacetals (Niemeyer et al., 1982). Rate-pH profiles showed that the active species were undissociated DIMBOA and thiolate anion. Higher nucleophilicity of thiolate anion, as reflected by higher pK_a of the parent thiol, enhanced its rate of reaction with DIMBOA. The analogue of DIMBOA lacking the 7-methoxy group reacted slower than DIMBOA. Finally, CNDO/2 molecular orbital calculations indicated that nucleophilic superdelocalizability, a measure of the tendency of an atom to interact with a nucleophile, was highest at the nitrogen atom. The following mechanism was proposed for the predominant reaction (Perez & Niemeyer, 1985):



The reactivity of DIMBOA towards a sulfhydryl group in an enzyme was studied using papain. This enzyme has a single free cysteine residue which is located at the active site. The enzyme was irreversibly inhibited by DIMBOA. Disappearance of titratable SH groups and of enzyme activity were synchronous. Inactivation by DIMBOA was protected by substrate and could be reverted by dithiothreitol. The rate of inactivation-pH profile showed 2 inflections corresponding to the 2 dissociation constants associated with the cysteine residue at the active site. HPLC analysis of the organic products of an equimolar mixture of DIMBOA and papain failed to show evidence for lactam or free DIMBOA, suggesting that DIMBOA remained attached to the inactivated enzyme. The DIMBOA analogue lacking the 2-hydroxy group, and hence unable to form an open-chain compound, showed no effect on enzyme activity. The following mechanism accounts for the experimental observations (Perez & Niemeyer, to be published):



Reaction of DIMBOA with amines. The main products of the reaction of DIMBOA with n-butylamine were the Schiff bases arising from nucleophilic addition to the aldehydic and hydroxamic carbonyl groups in the open-chain analogue of DIMBOA. Only the ϵ-amino group in lysine was reactive between pH 5 and 12. This was shown through the use of α- and ϵ-N-acetylated lysine as model compounds, and is in agreement with the higher nucleophilicity of the ϵ-amino group, as reflected by the higher pK_a of its conjugate acid.



Distribution of hydroxamic acids in wheat. A screening for Hx levels of **Triticum** species and their immediate **Aegilops** ancesters was carried out. Hx were found in all the accessions analyzed, concentrations varying within a wide range. Extreme values were observed in the diploid **Aegilops** and **Triticum** accessions. Interestingly, tetraploid wheats arising from two diploids with high Hx levels have not been described. This possibility might be worthwhile considering for the production of high Hx-level wheats (Niemeyer, to be published).

Acknowledgements

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EFFECTS OF HOST PLANTS ON SUSCEPTIBILITY OF LEPIDOPTERAN LARVAE TO INSECTICIDES

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1. Introduction

Many plants are thought to contain toxic chemicals for defence yet almost all species of wild and cultivated plants are attacked by some insects. To enable them to feed and survive upon toxic diets many phytophagous insects possess a wide range of potent detoxifying enzymes (Dowd et al., 1983). Of these the microsomal cytochrome-P450-dependent monooxygenase system is considered to have a vital role in the oxidative metabolism of a huge variety of xenobiotics (Hodgson, 1985), particularly in polyphagous species which may encounter a plethora of plant toxins. Very little attention has been given to other enzyme systems such as esterases which may also be involved in the metabolism of allelochemicals.

Likewise, pesticides may be metabolised to more polar compounds by a variety of detoxifying enzymes (Wilkinson, 1976) and resistance to insecticides is frequently associated with a rapid induction of these enzymes. It seems likely that allelochemicals in host plants may induce detoxifying enzymes in insects grazing upon them (Terriere, 1984) and this may in turn render the insect less susceptible to subsequently applied insecticides. This paper examines this possibility and includes some initial studies on monooxygenases in this insect.

2. Materials and methods

2.1 Insects

A laboratory strain of **Heliothis armigera**, susceptible to insecticides, and continuously reared in Reading under standard conditions (Ahmad & McCaffery, 1986) was used for these studies.

2.2 Diets

Unless otherwise stated insects were fed on a haricot bean artificial diet (Ahmad & McCaffery, 1986). Diets based on wheatgerm or alfalfa were adapted for use with **H. armigera**. For induction experiments 0.01% coumarin, 0.2% α-naphthyl acetate (α-NA) or 0.1% or 0.2% phenobarbital (PhB) were thoroughly mixed into the diet at the molten stage during preparation.

2.3 Insecticide bioassays

Susceptibility to insecticides was determined using topical application. Serial dilutions of technical grade materials were made and a 1 ul drop applied to the dorsal thorax. Between 20 and 40 larvae were treated at each dose and at least five doses of each insecticide were used.

Control insects were treated with acetone alone. 72h mortalities were analysed using probit analysis.

2.4 Monooxygenase studies

In initial studies, microsomes from various tissues of **H. armigera** larvae were prepared largely following the methods of Brattsten & Wilkinson (1973). Optimum incubation conditions, substrate concentrations and pH were determined (McCaffery, unpublished) and for the current studies N-dealkylase activity was measured using p-chloro N-methylaniline as substrate (Brattsten & Wilkinson, 1973). For later routine studies post-mitochondrial supernatants were used.

3. Results

3.1 Susceptibility to insecticides of larvae fed on various artificial diets

Larvae fed on a haricot bean diet were significantly less susceptible to topically applied **cis** cypermethrin than larvae fed a wheatgerm diet (Table 1). Larvae fed on an alfalfa diet were of intermediate susceptibility. Likewise larvae fed on the wheatgerm diet were around twice as susceptible to topically applied carbaryl as those fed on the haricot bean diet (Table 1).

Table 1. Susceptibility to insecticides of 3rd instar **Heliothis armigera** fed on various artificial diets. Results of probit analysis.

Diet	LD_{50} (µg/larva)	95% Fiducial limits Lower	Upper	LD_{90}	Slope	S.E.
Insecticide: cis cypermethrin						
Wheatgerm	0.12	0.06	0.19	1.58	1.15	0.23
Alfalfa	0.19	0.13	0.25	0.79	2.05	0.32
Haricot	0.20	0.15	0.25	0.98	1.86	0.22
Insecticide: carbaryl						
Wheatgerm	2.52	1.28	3.98	17.48	1.65	0.36
Haricot	5.14	3.14	8.01	53.58	1.27	0.29

3.2 Susceptibility to insecticides of 3rd instar larvae fed diets containing inducers of detoxifying enzymes

Insects on a diet fortified with the monooxygenase inducer coumarin were as equally susceptible to HCH and **trans** cypermethrin as control insects (Table 2). In contrast, larvae fed on coumarin required almost twice as much carbaryl to achieve an LD_{50} as those fed on the control diet. The inclusion of the esterase substrate α-naphtyl acetate in the diet did not protect 3rd instar larvae from the effects of **trans** cypermethrin (Table 2).

Phenobarbital (PhB) is a well known inducer of microsomal monooxygenases and it incorporated into diet at concentrations of 0.1% or 0.2%. Larvae fed on these diets required considerably greater quantities of

carbaryl to achieve comparable levels of mortality to those found with control insects (Table 3).

Insects fed on a diet containing coumarin required 7.5 times as much carbaryl to achieve an LD_{50} as those fed on a control diet (Table 4). This was a considerably greater tolerance than that found with third instar insects (1.9x Table 2). Similarly larvae fed on α-naphthyl acetate were over twice as tolerant at LD_{50} to **trans** cypermethrin as larvae fed on a control diet. At LD_{90} these insects required over 14 times as much insecticide to achieve a similar level of kill to that of control insects (Table 4).

Table 2. Susceptibility to insecticides of 3rd instar larvae of **Heliothis armigera** fed on artificial diets containing either 0.01% coumarin or 0.2% α-naphthyl acetate.

Treatment	LD_{50} (µg/larva)	95% Fiducial limits Lower	Upper	LD_{90}	Slope	S.E.
Carbaryl						
Control	3.90	2.64	6.32	>500.00	0.27	0.33
Coumarin	7.51	3.90	38.76	214.18	0.88	0.30
HCH						
Control	5.37	4.09	7.69	16.63	2.61	0.48
Coumarin	5.76	4.32	8.35	19.42	2.43	0.46
Trans cypermethrin						
Control	0.064	0.046	0.102	0.275	2.03	0.41
Coumarin	0.056	0.039	0.092	0.292	1.79	0.37
α-NA	0.056	0.039	0.078	0.260	1.92	0.34

Table 3. Susceptibility to carbaryl of 3rd instar larvae of **Heliothis armigera** fed on artificial diets containing 0.1% or 0.2% phenobarbital.

Treatment	LD_{50} (µg/larva)	95% Fiducial limits Lower	Upper	LD_{90}	Slope	S.E.
Control (W)	2.52	1.28	3.98	17.48	1.65	0.43
0.1% PhB	4.79	1.99	10.24	25.62	1.74	0.46
0.2% PhB	6.50	4.05	10.62	32.51	1.89	0.39

3.3 Detoxifying enzyme systems

Initial studies have concentrated on the microsomal monooxygenase system. Preparatory studies to determine reaction conditions, pH and substrate concentrations were carried out (see section 2.4). Table 5 shows the tisssue distribution of N-dealkylase activity in 6th instar larvae.

Table 4. Susceptibility to insecticides of 6th instar larvae of **Heliothis armigera** fed on artificial diets containing either coumarin or α-naphthyl acetate.

Treatment	LD_{50} (μg/larva)	95% Fiducial Limits Lower	Upper	LD_{90}	Slope	S.E.
Carbaryl						
Control	46.74	22.59	99.12	852.15	1.02	0.28
Coumarin	349.79	203.37	1395.90	2039.06	1.67	0.46
Trans cypermethrin						
Control	0.144	0.110	0.191	0.442	0.40	2.62
α-NA	0.311	0.163	0.554	6.220	0.99	0.18

Table 5. Tissue distribution of N-demethylase activity in 6th instar **Heliothis armigera.**

Tissue	N-demethylase activity (mean + S.E.M.) ($nmol.m^{-1}$. larval $equivalent^{-1}$)
Midgut	5.98 ± 0.25
Fatbody	5.11 ± 0.30
Malpighian tubules	0.21 ± 0.05
Integument	0.09 ± 0.02

Oxidase activity in both midgut and fatbody at various times during the 5th and 6th larval instars of **H. armigera** varies considerably as shown in Table 6. Highest activities were recorded during the middle of the 6th instar with negligible activities near the moults and during the pre-pupal stage.

Table 6. N-demethylase activity at various times during the 5th and 6th larval instars of **Heliothis armigera.**

Instar and stage		N-demethylase activity (mean + S.E.M.) ($nmol.m^{-1}$.larval $equivalent^{-1}$) Midgut	Fatbody
5	Mid	0.32 ± 0.25	0.26 ± 0.17
5	Late	0.04 ± 0.02	-
6	Early	0.48 ± 0.27	0.56 ± 0.21
6	Mid	3.72 ± 0.52	3.48 ± 0.66
6	Late	0.97 ± 0.13	0.82 ± 0.22
6	Prepupa	0.07 ± 0.02	0.15± 0.10

4. Discussion

These studies show that when larvae of **H. armigera** are fed on different diets they are susequently found to have differing levels of susceptibility to insecticides. This has been noted in a number of other lepidopteran larvae (e.g. Wood et al., 1981). The effects seen here are

clearly dependent on the host plant (or allelochemical) and insecticide combination used.

The enzyme systems which metabolise xenobiotics include the monooxygenase, esterases and transferases (Terriere, 1984). Presumably, the degree to which the host plant can influence the susceptibility of the insect to a subsequently applied insecticide depends on the degree of similarity between the enzymes metabolising the two xenobiotics. In this respect an allelochemical which is a good monooxygenase substrate would be expected to confer tolerance to a subsequently encountered monooxygenase substrate (assuming continued induction). This appears to be true for **H. armigera**. Coumarin is a plant compound which is metabolised by the monooxygenase system. Larvae fed on a diet containing sub-lethal quantities of coumarin are considerably less susceptible to carbaryl which is also metabolised by this enzyme system. Likewise, the monooxygenase inducer phenobarbital also confers tolerance to carbaryl.

HCH and **trans** cypermethrin are transferase and esterase substrates respectively, so coumarin ingestion does not confer any metabolic ability to deal with these materials. There is little or no evidence of induction of esterases by plant chemicals but clearly tolerance of **trans** cypermethrin is more than doubled following the inclusion of α-naphthyl acetate in the diet of 6th instar larvae.

The effects noted above are considerably more marked in 6th instar larvae than in 3rd instar larvae suggesting that the older insects are considerably more enzymically inducible than the younger insects. This is especially true of the possible esterase induction. Moreover, **N. demethylase** activity is significantly higher during the middle of the 6th instar than at other times confirming the above results. Preliminary studies with other monooxygenase forms indicate similar results and have also been found previously (Hodgson, 1985).

These effects are of especial importance in pest management. If certain crops are more potent inducers of detoxifying enzymes then clearly greater quantities of certain insecticides will be required. Some insecticides are rendered more toxic by these enzymes and the converse would be true. Further studies are essential.

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CHAPTER 2. INFLUENCE OF THE PLANT, HABITAT, COMMUNITY COMPOSITION, DISTRIBUTION OF PLANTS ON FORAGING BEHAVIOUR, ENTOMOPHAGOUS ACTIVITY AND STRUCTURE OF INSECT POPULATIONS AND COMMUNITIES:

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PLANT VARIETY AND ITS INTERACTION WITH HERBIVOROUS INSECTS

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The relationship between a herbivorous insect and its food plant may be classified according to the features of behaviour or biology of the insect involved. These may be viewed as the sequence that constitutes colonisation, namely: attraction, settling, tasting, feeding, digestion and/or the reproductive activities. Particular properties of the plant and often of the community (the biocenose) of which it is a part, will influence insect behaviour during these different stages. Plant-insect relationships have been evaluated within this framework, provided by insect behaviour, in several symposia (Labeyrie, 1977; Ahmad, 1983). In this introductory paper I will use another framework, that provided by the plant, i.e.:

a. Community features
b. Features of individual plants
c. Features of individual leaves
    i. Variations between species
    ii. Variations within species
        a. Intrinsic
        b. Due to other organisms

1. Community features - Spatial and temporal patterns

For a phytophagous insect a major determinant of the 'grain-size' of its habitat, the extent of its patchiness, is the distribution of its host plant. Large patches of plants may arise because many seeds of the same species germinate in a locality (or seedlings are planted by man) (e.g. trees in many northern forests: birch (**Betula**), spruce (**Picea**) or because the species has a modular form (Harper, 1977) and many so-called 'plants' arise from a single genet (seed, etc) (e.g. **Pteridium, Rubus, Lotus**).

In a study of secondary succession in Southern England colleagues and I found that patch-size increased with successional age of the community (Southwood et al., 1983). The combined effect of patch-size and patch frequency can be conveniently, and I believe meaningfully, expressed as the 'expectancy' of finding plants of the same type following random movement from one individual plant. Although this pattern holds in many Northern temperate regions for the dominant plants, it does not apply to the rarer species that may be very scattered in all communities.

Temporal patterns may also be looked at in the same terms - what is the expectancy of a plant being in the same place over a period of time? This has been termed its durational stability (Southwood, 1977). These

expectancies also increase with successional age (Southwood et al., 1983).

These patterns are the result of competitive processes between plants: the evolutionary pressure to compete for and 'hold-on' to space. The effects of these patterns on the relationships between the plants and their herbivores are distinct, depending on whether it is the plant or the insect that is being considered.

For the insect herbivore there are two processes: initial discovery and population development. As Stanton (1983) has argued for those insects whose movements are essentially random, larger patches with their greater peripheries will be discovered more frequently than small patches. (For insects that descend following carriage in winds in the air it may be the greater area, rather than periphery, that is important). For those insects that orientate by certain signals these are likely to be enhanced by a large patch of the host plant: such signals may be olfactory or visual (e.g. Kareiva, 1982). In fact the movement of insects between host plants usually consists of varying mixtures of random and directional movement (Kennedy, 1986).

That the chances of discovery and infestation are not simply a matter of the plant and its patch-size is evidenced by the many observations on the importance of the surrounding vegetation: plants growing amongst other species of plant are less likely to be discovered than those growing alone on bare ground (Pimentel, 1961; Root, 1973) (Table 1). Studies on airborne

Table 1. Effect of patch size and surroundings on probability of an oat shoot being infested by **Oscinella frit**. (Oats sown 8 April 1961, examined 4-8 June; unpublished data; work undertaken at Silwood Park, Berks, UK).

Patch size	Large (field)	Small (1 row)	Small (1 row)
Surroundings	Oats (same age)	Short grass	Bare ground
No. shoots examined	222	282	258
No. *O. frit* stages found	64	0	138
Probability of shoot being invaded	0.29	< 0.03	0.53

insects have shown that they do not avoid bare ground; indeed the aerial density a metre or so above bare ground, is high. Whether this is due to simple accumulation, because of the absence of vegetation on which to settle, or to some behavioural 'attraction' or to the higher surface temperatures causing the formation of a small scale convergence zone is unknown. But whatever the mechanism or combination of mechanisms, these relatively abundant potential colonists may be aided in their discovery of the isolated plant in bare ground by the clarity of recognition signals, such as the silhouette of the plant or the surface of the leaf. Rausher's (1978; Papaj & Rausher, 1983) studies on the pipe vine swallowtail butterfly, **Battus philenor** have shown how females recognise leaves of a particular form, this leads to settling, after which contact receptors confirm or refute the visual identification. Thus it is failure of the female to discover the host plant that is the primary cause of the lower rates of infestation by **B. philenor** larvae, of **Aristolochia reticulata** when this plant grows in dense natural vegetation or when, late in the season,

the height and density of the surrounding vegetation increases.

Observations of the type shown in the previous table led Root (1973) to propose the 'resource concentration hypothesis' which states that herbivores are more likely to find and remain on hosts that growing in dense or nearly pure stands. Although any plant species shows some natural variations in its patch size, outside this limited range major changes of scale in natural situations are due to different species and, as shown above, certain features of scale are associated with different seral stages. Insects have evolved tactics and strategies appropriate to their host plant's patch size. Janzen (1984) has contrasted the different bionomic strategies of tropical Saturines and Sphingids: both large moths, but the former have host plants that occur in large patches (relative to the moth) and are long lived, whilst the reverse is true for the hawk moths larval food plants. He shows how many differences in adult longevity, feeding, flight and defence tactics may be related to the features of the host plant.

Now to consider these features from the evolutionary view point of the plant. One might postulate theoretically, using arguments analagous to those of Hamilton (1971), that the individual plant has less chance of being discovered in a large patch than if isolated. However unlike predation in animals (considered by Hamilton), herbivory is a continuing process - in some ways resembling parasitism; herbivores are likely to spread from one plant to its neighbours. Some plants have, under this and/or other evolutionary pressures evolved a life strategy of single, scattered individuals (e.g. **Aristolochia**). Others, termed competitors by Grime (1977), form large patches as part of their strategy in inter-plant competition. This patchiness has, as shown by Southwood et al. (1983), both spatial and temporal dimensions: the ruderal plant (e.g. **Capsella bursa-pastoris**) lives for a few months and has a low durational stability for any insect, but at the other extreme many trees will permit hundreds of generations of insects (even those with annual life-cycles) to pass in the same location. Feeny (1976) and Rhoades & Cates (1976) distinguished the two strategies as non-apparent, plants that are scattered in space and time, and apparent, plants whose spatial and/or temporal exposures (and hence expectancies of being found) are high. Of course all plants are to some extent apparent, but this may be reduced by spatial and temporal variation within the plant: the effect of this on herbivory load will be explored later.

It is easy to assume that herbivory is detrimental to the fitness of the plant and although this is true in the general sense (as shown by Waloff & Richards', 1977, data), it is by no means true for every herbivore all the time. A recent study by Cottam et al. (1986) has shown how the effects of beetle herbivory on a dock (**Rumex obtusifolius**) are related to the extent to which the plant is competing with grasses. Grazing had little impact on the growth of non-competing plants, but significantly reduced that of those that were competing. This synergism between competition and grazing, long recognised for vertebrate herbivores (Harper, 1977), is important when considering the impact of herbivory on plants. The cost, in terms of inclusive fitness, of herbivory is thus greater to an isolated

dock genet growing amongst grass, than to a shoot (module) of grass or bracken growing in a large patch of genetically identical plants. This would provide an evolutionary advantage for large patch size notwithstanding the higher expectancy of discovery discussed above.

2. Features on individual plants - structural complexity of life forms

The structual complexity of a plant - the variety of its forms (architectural complexity) and their distribution in space (spatial complexity) is positively correlated with the size and diversity of its insect fauna (Lawton & Schroder, 1977). Southwood et al. (1979) showed how in a successional gradient, the increasing diversity of the insect fauna of certain groups could be largely explained by the increase in structural diversity in the older seres, whilst Stinson & Brown (1982) showed that 79% of the variance in leafhopper (Homoptera Auchenorrhyncha) species richness could be accounted for by variation in the architectural complexity of the grasses in the different communities. The relative contributions of size (spatial complexity) and design variation (architectural complexity) on species richness was investigated for **Opuntia** cactus by Moran (1980), who found that size alone accounted for only 35% of the variation in species richness, whilst all competents of structural complexity together accounted for 69%. Fowler (1985) found that tree size alone had little impact on species richness of herbivores on birch (B**etula**). But whatever the importance of the different components we see that the evolution of the large, diversely structured, life-forms, characteristic of the dominant plant species for many associations, is associated with increased exposure to a variety of herbivores.

3. Features of individual leaves

(I am confining my discussion to leaves, not because stems, roots and reproductive structures are unimportant, but because of the constraints of space and time).

3.1 Variations between species

Recognising that various features of a leaf, both physical and chemical, contributed to its palatability to insect herbivores and that this was different in different plants Feeny (1976) and Rhoades & Cates (1976) proposed the theory of apparency referred to above. They postulated that early successional plants might escape herbivore damage in space and time. In an extensive study of tropical trees, Coley (1983) found that foliage of 'gap species' was heavily damaged and that herbivory rates were not related to apparency. Later (Coley et al., 1985) she proposed a new theory: resource availability to the plant determined its leaf palatability. In a comprehensive study on the organisms in a series of seres in a secondary succession (to which I have referred already) colleagues and I have obtained data on palatability from a bioassay by generalist herbivores (Reader & Southwood, 1981), life expectancy of leaves and damage levels in the field (Southwood et al., 1986). These data have been obtained for plant species with a wide range of life forms, from short-lived ruderals to trees. We found that palatability to insect

herbivores was strongly correlated with life-expectancy of the leaves (r = 0.85), but the amount of damage suffered in the field was inversely correlated with palatability. S. Greenwood (pers. comm.) has obtained rather similar patterns along a disturbance gradient into a tropical forest in the Far East, whilst Rathcke (1985) found with slugs that acceptability of foliage declined with longer-lived leaves (seasonal persistence). Thus though the rate of damage was less on the less palatable leaves, their increased longevity means that they accumulate damage over a longer period so total damage may be more. Whether it will be more, as we found with oak (**Quercus**), or less, as we found with holly (**Ilex**) and Coley (1983) found with her shade tolerant tropical forest trees, depends on the balance between palatability and the lenght of life of the leaf. Our findings therefore give some support to both 'apparency theory' and 'resource availability theory' and suggest that these theories correspond with different axes of the habitat templet (Southwood et al., 1985). The demonstration by Cottam et al. (1985) that insect herbivory has a synergestic effect with competition provides evidence for the evolutionary pressure to increase the commitment to defence as competition increases, and as favourableness and resource availability falls.

3.2 Variations within species

These can be further subdivided into those that are entirely dependent on the plant and those that are at least in part due to other organisms.

The leaves of the same plant may vary in their features with age, with season, and with position on the plant; within a species of plant they can vary from one site to another. The fitness of the plant will be increased if herbivory falls most heavily on those leaves that are of least value to it. The value of a normal leaf to a plant resides primarily in two functions:

a) as photosynthetic factories and store houses

b) as weapons in competition with other plants.

The relative importance of the roles may vary with age and position of the leaf. There is some evidence that in trees productivity is greatest in the first part of the summer season and is heavily dependent on the outer layer of leaves. The importance of many of the leaves in the inner part of the crown and of most leaves in the early autumn is probably to cast a deep shade and so hamper growth of competing seedlings. Some oaks (**Quercus**) produce an additional flush of leaves in late summer, lammas growth: the primary advantage of this may be to deepen the competitive shade, rather than to increase productivity.

Whatever their impotance, and usually this is considerable, young leaves are relatively undefended for a tough cuticle and low water contents, important components of palatability (Coley, 1983b), are not compatible with rapid expansion and high metabolic activity. Secondary plant substances generally, but not invariably, accumulate after the leaf has expanded (McKey, 1979). Feeny's (1970) original observations on changes in tannin levels on oak leaves with season lead to the conclusion that seasonal feeding patterns of lepidopterous larvae on oak vere evolved to avoid high levels of tannins later in the season. Certainly larvae

develop more slowly (one cost of which is increased exposure to predators) and often achieve a lower pupal weight (producing less fecund females) than those emerging early in the season (Feeny, 1970; Wint, 1983; West, 1985). But it may be the falling level of protein, rather than tannin levels, that is the cause of this decrease in food quality. Questions have been raised about the precise effects of tannins in herbivores (e.g. Berenbaum, 1980; Bernays, 1981) and on the appropriateness of methods of analysis and quantification (A. Wilson, unpublished). Others at this meeting will explore further the enigmatic role of plant secondary substances in anti-herbivore strategies.

Since the classic work of J.S. Kennedy showing that aphids have a preference for young and senescing foliage, many workers have demonstrated the importance to the herbivore of leaves of a particular age, condition or position. For example: most **Heliconius** larvae feed only on young growing **Passiflora** shoots (Gilbert, 1977), the bug **Leptopterna dolabrata** feeds selectively on the growing shoots and flowering heads of the grass **Holcus** (McNeill, 1973) and higher numbers of the leaf hopper **Ribautiana ulmi** fed on the more highly illuminated leaves of **Ulmus montana** than the shaded ones though the former had tougher leaves and lower water contents (Claridge, 1985). If the arguments I have advanced are valid, these latter observations represent the failure of the tactics of the plant to shift the herbivory load to the less valuable shaded leaves.

The importance of particular parts of plants for insect herbivores has had a profound influence on their behaviour and life-histories. The evolution of the long life of the adult female **Heliconius** and her behavioural and feeding traits have allowed her to exploit the temporally and spatially scattered growing shoots of **Passiflora** as oviposition sites (Gilbert, 1977). Moore (1986) has shown that some tropical Satyrines, particularly **Mycalesis perseus**, select young and nutrient rich grass shoots for oviposition, whilst cool temperate species lay indiscriminately. A number of factors may contribute to this difference: the temperature limited flight-time of cool temperate butterflies (females often fail to lay all their eggs and cannot 'afford' to spend time searching), the rapid and short growing season of some tropical grasses so that wandering larvae might be too late and the greater risk in the tropics from predators (expecially ants) to a larva searching for its host grass. But all these factors depend on the larval requirement for particular grass shoots. Adult viburnum whiteflies (**Aleurotrachelus jelinekii**) mostly fly only a few centimetres from the year old evergreen leaf on which the lived as larvae, to a young leaf for oviposition; although the leaves of **Viburnum tinus** generally live for 2 to 3 years the insects redistribute themselves in this way placing their young where soluble nitrogen levels are highest (in newly expanded leaves) (Reader & Southwood, unpublished).

Since the work of Haukioja & Niemela (1979) it has been recognised that feeding by herbivores may modify the composition of the leaf, in ways not completely understood, but sufficient to modify its value to other herbivores. This is clearly the basis for the host plant mediated interspecific competition demonstrated on oak between the early folivorous caterpillars (especially **Operophtera brumata**) and the oaf tortrix (**Tortrix**

viridana) and leaf-miners and sap suckers that feed later in the season (West, 1985; I. Silva, unpub.; M. Hunter, unpub.). West's work shows how to an ovipositing female leaf miner (**Phyllonorycter**) an oak tree is a mosaic of regions of different favourability overlain, on a leaf to leaf scale, by the level of damage a leaf has already sustained: reproductive success depends on the selection of the optimum oviposition sites from this mosaic.

This review has many omissions, for example, nothing has been said about the variations in the surface structure of the plant, both between and within species (Juniper & Southwood, 1986). But this survey has shown how the great variety of plant form and composition offers a diversity of different challenges to herbivorous insects and as labeyrie (1977) has stressed, it is on this complex set of signals from its own ecosystem that selection has occurred to identify the correct signals.

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DIET OF THE ADULTS OF ACANTHOSCELIDES OBTECTUS AND ITS EFFECT ON THE SPATIAL PATTERN OF THE ATTACKS IN THE FIELDS OF PHASEOLUS VULGARIS

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1. Introduction

Many insect species consume pollen and nectar. However, except for the bees, very little is known about the use of pollen by insects. On the other hand, papers on pollination by insects are numerous (Stanley & Linskens, 1974).

As far as polyvoltin bruchids colonizing stored grain legumes are concerned, the importance of adult feeding in the fields has been drastically underestimated.

It is obvious that adults are able to survive without feeding, because the larvae accumulate reserves during their development in the stored grains. However, many studies led to the conclusion that longevity and fecundity can be drastically increased when water, sugars and/or bee pollen are offered to them. Moreover, the enzymes of the digestive tract are well correlated with the nutrients available in the pollen grains (Leroi et al., 1984).

A. obtectus has rarely been observed on different flower species (Labeyrie & Maison, 1953; Zacher, 1951; Hase, 1953; Zachariae, 1958). Our own observations (Jarry, 1984) confirmed that numerous adults visit weed flowers present in fields or nearby. In this paper we intend to:

1) present the specific diet of the adults of **A. obtectus** by the analysis of the digestive tract content of adults captured in a plot of **P. vulgaris;**
2) analyse the effect of the food location (weeds) on the attacks of the bean pods by comparing the attacks in weeded and unweeded plots.

2. Materials and methods

We captured the adults on climbing beans at Aire sur l'Adour (Landes) from the 1st of august to the 18th of september 1981, and then kept them in 70% alcohol. Dissections were made in the laboratory, on a microscope slide where the digestive tract was scratched and the content diluted in a drop of 70% alcohol, then fixed in glycerined gelatine. Following a first separation, the slides containing more than 50 identifiable pollen grains were analysed more precisely: thus the different types of pollen were identified under high magnification (x 400) in the region with highest density and the census was made at a medium magnification (x 200) by a longitudinal screening.

The attacks were estimated in 1981 at Aire sur l'Adour in a plot of dwarf beans (8 rows x 32 plants). The distance between the rows and between

plants was 0.80 m. Sixteen sub-plots (weeded and unweeded) were distributed according to a design in check pattern (Fig. 2). After the harvest, all the rippen pods were kept at the temperature of the laboratory till the emergence of the adults. The number of holes per grain were counted. Data from each pod were pooled, plant or group of plants according to their location in the plot. Maps of the results were analysed with the index of Geary (Chessel, 1981).

3. Results

Pollen analysis. We examined 457 adults (116 males and 341 females). Different groups have ben identified after dissection: (1) digestive tracts apparently empty (131), (2) those containing digestive left overs in the posterior part of the intestinal tract (105), (3) those more or less full which were mounted on slide (221).

144 slides (28 males and 116 females) were kept for analysis. On the others we found: (1) numbers of spores and/or pollen too little to be counted, (2) different aggregates preventing the counting, (3) some liquid more or less viscous without identifiable elements. This liquid has been also observed in the slides analysed. Out of the 144 slides, 9300 spores were counted in 70% of them, and 33600 pollen grains from the totality. We did not identify precisely the spores which were either monocellular, septated, or under a cyst form.

Table 1: Pollen found in the digestive tract countent of 144 adults of **A. obtectus** captured in a bean plot (between brackets, the number of slides where the type was observed).

Type of pollen	Percentage compared to the total number of pollen grains counted		
	males	females	total
Non cultivated grasses	55.39 (17)	63.13 (92)	62.53 (109)
Chenopodiaceae/Amarantaceae	17.99 (6)	21.20 (47)	20.95 (53)
Umbelliferae	11.76 (9)	5.55 (20)	6.04 (29)
Galium	4.24 (6)	2.59 (31)	2.72 (37)
Compositae type Achillea	3.36 (2)	2.39 (8)	2.46 (10)
Compositae type "échinulées"	2.44 (2)	1.56 (20)	1.63 (22)
Compositae type "fenestrées"	(0)	1.63 (4)	1.51 (4)
Polygonaceae	.11 (1)	.47 (5)	.44 (6)
Rumex	3.40 (2)	.03 (1)	.29 (3)
Lythrum	(0)	.23 (3)	.21 (3)
Centaurea	.23 (1)	.12 (3)	.13 (4)
Limonium	(0)	.13 (1)	.12 (1)
Phascolus vulgaris	(0)	.10 (3)	.09 (3)
Betula	(0)	.03 (1)	.03 (1)
Corylus	(0)	.02 (1)	.02 (1)
Zea mays	.04 (1)	.006 (1)	.009 (2)
Artemisia	(0)	.003 (1)	.003 (1)
Plantago	(0)	.003 (1)	.003 (1)
Undetermined	1.03 (6)	.82 (43)	.83 (49)

To find one unique type of pollen is rare (6%). More frequently a mixture between different types is observed (Table 1), in which one form dominates: non cultivated grasses, then Chenopodiaceae/Amarantaceae and Umbelliferae. These 3 types accounted for 90% of the total pollen grains counted. As far as rare types (< = .10) were concerned, we need to be very carefull: so, dissections were made on the slides, suplementary contaminations (**Betula, Corylus...**) or pollen grains existing on the insect itself were always possible. Small pollen (diameter < 30 u) was scarce, and **P. vulgaris** is poorly represented.

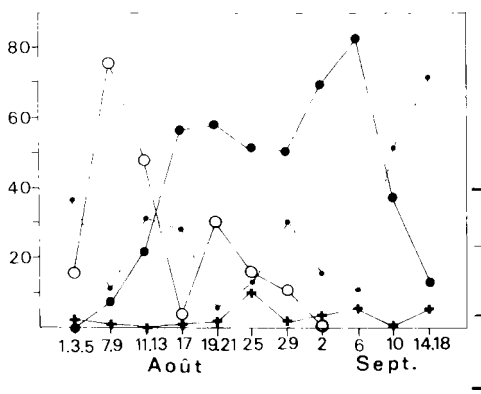

Figure 1. Fluctuations of the spores and pollen composition of the digestive tract of adults of **A. obtectus** according to their capture date.

Pollen composition varied according to time. Thus Chenopodiaceae/Amarantaceae, were dominant in the first period of the captures, and then were replaced by grasses, then spores (Fig. 1).

The frequency of slides retained for analysis of the content of the digestive tract was greater in females (116/341 = 0.34) than in males (28/116 = 0.24) (X^2 = 4.86, dF = 1, p = 0.03). It, therefore, results in the total values being closer to the female composition. Nevertheless males and females clearly show a similar composition of pollen types. A detailed analysis of the contents of the male digestive tract has not been possible because too few were collected; samples were taken at times when females outnumbered males and very few slides could be analysed when the reverse was true (Jarry, 1984).

Spatial pattern of attacks in a plot of dwarf beans.

We will not discuss in details here the problem of choosing an appropriate index of attacks (see Parfait, 1986). We have chosen the frequency of pods attacked per plant. Results were mapped (Fig. 2). Two levels of structure appear after the overall analysis of all the plots (matrix 8 x 32):

(1) the four rows near the hedge and the four others;

(2) a scale 4 x 4 corresponding to the sub-plots sizes.

So, the mean frequency of attacks per sub-plots gives a good representation of the results (Fig. 3).

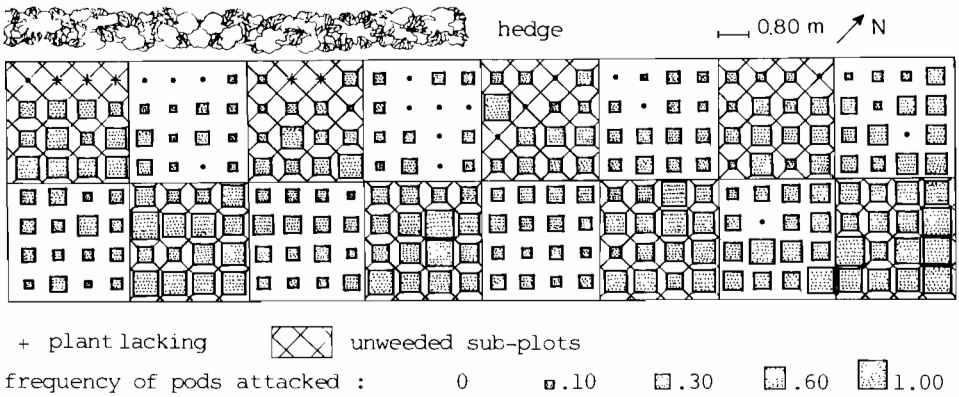

Figure 2. Spatial pattern of frequency of pods attacked per plant of **P. vulgaris.**

.36	.09	.25	.09	.24	.16	.25	.27
.19	.51	.21	.49	.21	.46	.37	.63

Figure 3. Mean frequency of pods of **P. vulgaris** attacked in each sub-plot (same legends that for figure 2).

Because of the first level of stucturation of the attacks in the fiels, cutting it in 2 parts, the frequencies will be compared separately in each half of the plot: results were clear enough: except one, unweeded sub-plots are always more attacked than the weeded ones.

4. Discussion

These results are quite surprising: despite the fact that grasses have a typical anemophylic pollination, their pollen is the main food source for the adults of **A. obtectus.** How can adults feed on this pollen, knowing that no adult were observed on grass flowers? Several authors indicate that adults eat the chlorophyll-rich tissues of bean. This was not confirmed by our analysis. But it is possible that adults lick the surface of the leaves, on which many wind-dispersed pollen grains are "trapped".

We analysed a sample of leaves collected in this plot (Jarry, 1984). Grasses and Chenopodiacaea are the dominant types, and the results indicate which kinds of weed exist near the plot (in particular **Echinocloa crus-galli** and **Chenopodium album**). At the opposite, numerous types (e.g. **Rumex, Plantago...**) which exist on the leaves are found in very limited amount in the digestive tract. Are the adults able to separate the different types of pollen on the leaves? Carefull experimentation is needed to answer this question.

Anyway, these results suggest that some weeds, whose spectrum still needs to be established, can be used as feeding sites by the bruchids. This could strongly influence the spatial pattern of attacks in the field. Numerous points remain to be clarified, such as the pattern of movements between feeding areas and egg-laying sites.

These results show that feeding of the insect is certainly more complex than thought. In the case of the herbivorous insects, much interest has been given to the damaged stages. But, larvae and adults of holometabolous insects frequently have very different diets. The example of the feeding behaviour of young spiders catching pollen in their webs (Smith & Mommsen, 1984), even if it is far from the insect/plant relationships illustrates very well this complexity.

Besides, feeding should not only be investigated from the point of view of insect physiology, but also has an important component in the structuration of the biocenosis. One typical example of this, is the celeri fly which feeds on pollen and nectar in the trees surrounding fields of the host-plant (Leroi, 1977). Understanding the structure of these insect populations requires an approach that goes beyond the boundaries of the experimental plots. In other words, this sets up the problem of how choosing the appropriate scale of study, when investigating the biology of natural populations.

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SPATIAL DISTRIBUTION OF CHESTNUT WEEVIL Balaninus (= Curculio) elephas POPULATIONS

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1. Introduction

In population biology, it is important to know how the individuals of a species are distributed in their habitat (Legay & Debouzie, 1985). In classical studies spatial structures are defined on the basis of indexes, such as the index of dispersion, or on the basis of relationships between variance and mean (Taylor, 1961, 1984; Iwao, 1968). If the practical usefulness of this approach must be underlined, its theoretical consequences on the spatial distribution are rather misleading since distributions are classed as "contagious" in 95% of the field studies.

Chessel and Debouzie (Chessel, 1978; Debouzie et al., 1975) have proposed to abandon random sampling and replace it with systematic designs. In the same papers Chessel introduces non parametric statistical tests well fitted to data collected by systematic sampling. These tests can detect several scales of heterogeneity within the area studied; we can discover true aggregativeness, that is, groupings of individuals in a sampling unit while only few individuals are found in the nearest one; we can also show gradients or local concentrations. The tests are complemented by graphical presentation of data (Debouzie & Thioulouse, 1986).

These ideas have been applied to intra-population distribution in the chestnut weevil **Balaninus (= Curculio) elephas.** In France the chestnut weevil has only one generation per year, one major host plant: the chestnut, and low mobility like other curculios (Ulmer et al., 1983). So, in most cases the whole development occurs on or under the same tree: adults emerge beneath a chestnut tree and fly up into it, where females deposit eggs in the fruit. The larvae develop in a chestnut, crawl outside it after it falls, then burrow into the soil beneath the same tree. The peculiar life history of the chestnut weevil gives a good opportunity to study **the spatial relationships between insects and their host plant,** and to show that variations in the relationships can occur on a very fine scale.

In this paper, we attempt to answer the following questions:
- are chestnuts and weevil larvae randomly distributed in each stand?
- why are some trees heavily infested while others are not attacked?
- can we test hypotheses proposed to explain the observed variations?

2. Materials and methods

These studies were conducted in seven mature stands of European chestnut **Castanea sativa** near Lyon at St-Just Chaleyssin (Isère, France).

An area less than 1 km^2 was studied; it contains eleven old wild chestnut trees. The stands include either one isolated tree or several contiguous trees. Under normal meteorological conditions, the area is, as far as the weevils are concerned, effectively isolated from other chestnut stands in the vicinity.

Data have been collected since 1981. Each stand is divided into a grid of plots, each 5 m x 5 m. Depending on the size of stands, the number of plots varies from 13 to 38. One 0.5 m^2 sample of fallen chestnut fruit is taken from the center of each plot. On the average 12 samples are collected from the fall of the first chestnuts in late September to early November. Chestnuts are opened in the laboratory and the number of weevil eggs, larvae or exit holes found is recorded.

30 box emergence traps, 1 m^2, are placed beneath the trees for monitoring chestnut curculio emergence. The number of adults is recorded from the first emergence, in mid-August until the end of September.

The number of larvae and adults per stand is estimated by extrapolating the value found in chestnut samples or in traps to the whole stand.

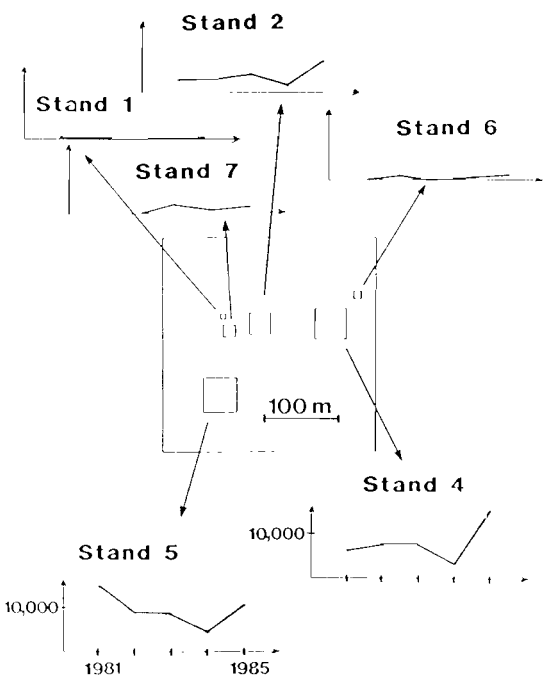

Figure 1. Numbers of chestnut weevil larvae estimated in each stand. Curves give the estimation for each year since 1981 and for each stand. The central square represents the area studied. Each small square has a size in relation to the average number of larvae estimated from 1981 to 1985. Each also has the coordinates of the corresponding stand.

Choice experiments were done by placing a curculio a 7 to 14 day-old female in a cage (30 cm x 40 cm x 50 cm) with two twigs bearing chestnuts. Twigs are cut from stands 1 and 5; ones with only one bur are chosen; they were enclosed in 1 mm^2 mesh nylon bags to exclude potential immigrants. Females were caught in stand 4. Each experiment lasts 24 hours; the chestnuts are then opened and the number of eggs laid is recorded. 24 replicas have been done in September, 1985.

3. Results

3.1 Inter-stand variation in the abundance of chestnut weevils

The dynamics of chestnut weevil larvae for each stand, from 1981 to 1985, is given in Figure 1. We note the relative stability in the numbers estimated for each stand. For example in the most heavily attacked stand (stand 5), the number of larvae varies from 4,250 to 14,300; the infestation level varies from 14 to 30% but it does not depend on the number of available chestnuts.

Chestnut weevils are not evenly distributed among the seven stands. Three stands are almost not attacked (stands 1,3 and 6) and about 75% of weevil larvae are found in only two stands: 4 and 5. Similar results are observed with the adults: for example in 1985, 72% of the adults emerging from traps were caught in stands 4 and 5.

3.2 Intra-stand variation

Two stands (2 and 4) have several chestnut trees, 2 and 4 respectively. Intra-stand variation in the abundance of weevils is observed, specially in stand 4 (Fig. 2). Variation occurs at a very fine scale since the size of stand 4 does not exceed 50 m x 30 m. The observed gradient, decreasing from west to east, corresponds to different chestnut trees: the western tree contains most larvae and adults. The gradient has been observed each year since 1981; its intensity depends on the total number of weevils in the stand.

Thus, stands with several trees are heterogenous; **the relationships between the chestnut weevil and its host plant must be studied at the scale of one tree.**

3.3 Infestation rate and time of maturation of chestnut

Nuts in the same tree do not mature at the same time; usually they fall down during a three week period. But on the average some trees are early, with most chestnuts falling in the second fortnight of September (stands 1, 5, 6 and one tree in 4) while the other trees are late with most fruit falling in mid-October. Of course the precise timing varies from year to year, a difference of about three weeks exists between the most temporarity trees.

Capture-recapture experiments have shown (Debouzie et al., 1985) that inter-stand dispersal of adults is very low, less than 5% , since no exchange between stands 4 and 5 were observed. Thus, if we assume that most curculios, that is more than 95%, develop in the same stand during many generations (more than 100), it is interesting to know if curculios have adapted their developmental rate to that of the chestnut.

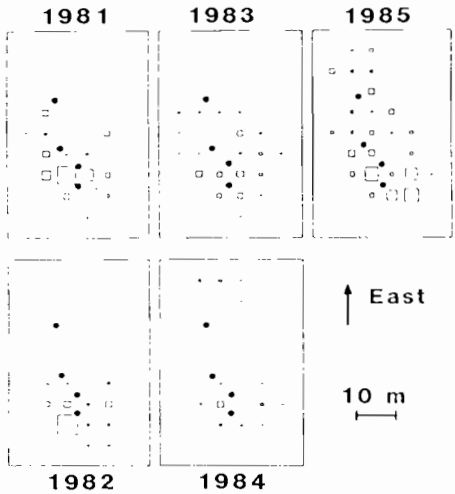

Figure 2. Maps giving the numbers of chestnut weevil larvae in a 4-chestnut -tree stand (stand 4). Squares have a size in relation to the total numbers of larvae found from the first fall of chestnuts to the time when they cease falling. Each sample is taken at the center of a 25 m square plot. Dark circles represent the four tree trunks.

Since emergence and consequently oviposition are spread over five weeks (Debouzie et al., op. cit.), developmental rate of larvae is very difficult to estimate. So, as a rough approximation, we propose to measure it by the proportion of exit holes made by the larvae which have finished their development. Whatever the year the conclusion is the same: no correlation can be found between the proportion of holes and the time of fruit maturation.

The timing of larval development does not differ regardless of whether the ripening of chestnuts is early or late; there is no synchronization with individual trees.

3.4 Choice experiments

Experiments were done to answer the question: why is the tree in stand 1 not attacked while that in stand 5, which is nearby heavily infested?

Preliminary experiments have shown that females restricted by a nylon bag can lay eggs in chestnuts of stand 1; the absence of high numbers of weevils in this stand cannot be explained by mechanical obstacles due for instance to the length or the density of the fruit thorns (Burges & Gal, 1981). Females can lay eggs in stand 1 but usually they do not.

Choice experiments were done to test the preference of females between chestnuts of stand 5 and those in stand 1. Among the 24 replicates 9 must be discarded because no egg laying was observed. Chestnuts of stand 5 were preferred in 12 replicates, chestnuts of stand 1 never, and we noted eggs in chestnuts of both stands in 3 replicates. Thus chestnuts in stand 5 are preferred but the choice is not absolute.

3.5 Interactions between chestnut and insects

The moth **Laspeyresia splendana** also attacks the chestnut. Its interaction with the curculio **B. elephas** are not yet understood.

Separate infestation levels may be estimated for each insect. We can compare, using the sign test, the observed frequency of chestnuts attacked by both insects and the expected frequency computed as the product of the infestation levels for each insect. Of 26 replicates available, 25 had observed values lower than the expected value ($N = 26$, $P < 10^{-6}$). So this test suggest a negative overall interaction between the two pests; the antagonism is expressed at the scale of the chestnut. However, no insect exclusion is observed: the observed value of simultaneous infestation by moths and weevils is much lower than expected by chance, but not null.

We can also compare the observed infestation levels estimated for each insect in each tree (Figure 3). A negative correlation ($r = -0.45$; $N = 27$; $P = 0.02$) exists between the numbers of moths and those of curculios. Thus, when a chestnut tree is attacked by many moths (about 50-60%), few or no curculios are observed; but it is also possible that moths are abundant because weevils are rare. Inversely, when many weevils are present in a tree (about 20%), fewer moths are observed (about 20-30%).

These two statistically independent approaches give similar conclusions but do not specify the true mechanisms of interaction. Preliminary experiments suggest that curculio females avoid laying their eggs in fruit already infested by moth larvae. But moth oviposition does not seem to be impeded by presence of eggs of larvae of weevils. So the interactions should not be symmetrical; nevertheless more experiments are needed to explain the observed results.

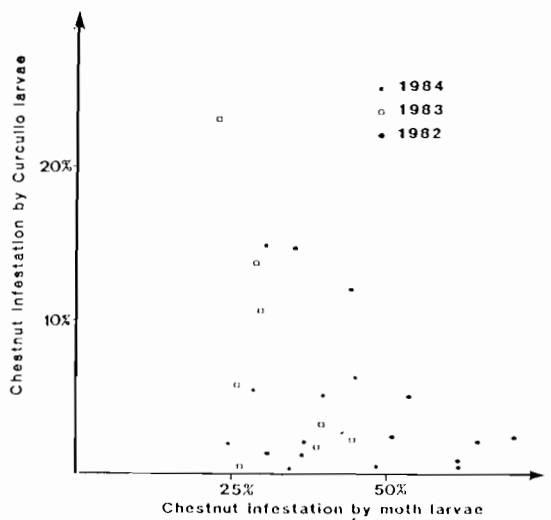

Figure 3. Infestation rates by weevil and moth larvae

4. Conclusions

Variations in the infestation levels are observed at a very fine scale since large differences exist between chestnut trees less than 100 meters away. Chestnuts of these trees are morphologically different but varieties or cultivars cannot be defined; the fruits are small, highly variable in a tree, like wild ones. Differences in the allelochemical products produced by them probably exist between the trees.

Thus, experiments must take into account spatial variation in the relationships between herbivores and their host plant. Similar results were obtained with cockchafer adults (Chessel et al., 1984), the Colorado beetle (Legay & Chessel, 1977), bark beetles (Coulson, 1979), and the checkerspot butterfly **Euphydryas editha** (Ehrlich, 1984). Moreover, interactions among herbivore insects attacking the same plant must be studied since the behaviour of an insect cannot be separated from its environment.

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INTERSPECIFIC COMPETITION DURING HOST PLANT SELECTION BY INSECT PESTS OF CRUCIFEROUS CROPS

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1. Introduction

Biologically-derived chemicals that influence insect behaviour are of great interest for broadening the scope of pest control in an acceptable manner. The presence of one pest species on a plant may influence whether another will select and successfully colonize that plant. The insect already present secretes or excretes repellent, alarm or warning chemicals which signal its presence to others. Few studies had been made of intra-specific behavioural relationships during host-plant selection (Rothschild & Schoonhoven, 1977) and even fewer on interspecific relationships. There is little quantitative data on this latter subject with respect to the pest complex on crucifers.

In 1982, Host-plant selection by the fly **Delia radicum** was altered when plants were already colonized by other insects (Finch, 1983). The fly avoided plants with caterpillars or frass of **Evergestis forficalis**, suggesting that these produced one or more repellent chemicals. This paper describes how host-plant selection by **D. radicum** is influenced by chemicals from frass and secretions produced by seven insects that also feed on cruciferous plants.

2. Materials and methods

Plants. All plants used in these experiments were grown from seed in 7.5 cm diam. pots of John Innes compost. They were maintained under controlled glasshouse conditions (days temperature 20°C, night temperature 10°C) and used for experiments when 8-10 weeks old. The plants were **Brassica oleracea** var **botrytis** L. (cauliflower), **B. oleracea** var **gemmifera** Zenk. (Brussels sprouts), **B. oleracea** var. **capitata** (L.) Alef (cabbage), **B. napus** L. var **napobrassica** DC. (L.) Reichenb (swede), **Raphanus sativus** L. var **longipinnatus** Bailey (Chinese radish), **Armoracia rusticana** Gaertn., May & Scherb (Horse-radish), **Alliaria petiolata** (Bieb.) Cavara & Grande (Garlic mustard) and **Cheiranthus cheiri** L. (wallflower).

Insects. All insects were reared, and all tests were carried out, in rooms illuminated for 18 h/day and maintained at 20 ±1°C and 65 ±5% r.h. Insects used included the aphid **Brevicoryne brassicae** (L.), the fly **Delia radicum** (L.), the beetle **Phaedon cochleariae** (Fab.), the butterflies **Pieris brassicae** (L.) and **P. rapae** (L.) and the three moths **Evergestis forficalis** (L.), **Plutella xylostella** (L.) and **Mamestra brassicae** (L.).

Delia was chosen as the test insect for the initial experiments because, unlike the other species 1) it lays in the soil alongside the selected plant rather than on the plant's foliage, 2) its eggs can be separated easily from the soil by flotation and 3) oviposition by one female does not deter oviposition by others.

Delia were reared on swede 'roots'. The other species were reared on various host-plants so that frass could be collected from the same insect species feeding on different host plants.

Preparation of experimental sprays. Frass was collected from filter paper placed beneath foliage on which insects were feeding. Suspensions for spraying were made by stirring 1 g of frass, or 1 g of macerated leaf tissue, into 50 ml of distilled water and then adding two drops of a wetting agent. All test plants were sprayed with 4 ml of either distilled water (control), frass suspension or macerated leaf suspension.

Tests involving sprays of frass and plant extracts. Potted cauliflower plants were used as the test plants in all experiments. Before being exposed to flies, the compost in each pot was covered with silver sand to standardize the oviposition medium. In each experiment, three plants were sprayed with water and three with either frass or leaf macerate. Test plants were exposed to the flies in a three-tier test chamber (Ellis & Hardam, 1975) 65 x 65 cm and 135 cm high. Each tier contained a 60 cm diameter turntable which rotated once every 4 min. Test plants were arranged symmetrically, though in random order, on the turntables.

Twenty male and twenty gravid female 5-6 day old **Delia** were released into each test cage and allowed to lay for 1 day. The plants were then removed and the eggs counted. Each experiment was repeated three times to produce nine replicates for each treatment.

Extraction of volatile chemicals from frass and plant leaves. **Frass:** 5 g of fresh frass was macerated for 1 min in 5 ml di-chloro-methane (DCM) containing 500 ug of pentadecane (C_{15}), as an internal standard. The mixture was then centrifuged and the lower DCM layer was collected. **Plant leaves:** 5 g of leaf material was macerated for 1 min in 10 ml of water. The macerated material was allowed to hydrolyse for 1 h at room temperature (Cole, 1980) and then extracted with DCM as for frass.

Analysis of extracts. Samples were analysed using a Perkin-Elmer Sigma 3 gas chromatograph fitted with a 10 m long, 0.53 mm internal diameter wide-bore capillary column filled with CP-Sil-5 CB. A Perkin-Elmer automatic injector transferred 1 ul samples of the various extracts into the chromatograph for analysis. The column temperature started at 100°C, rose by 10°C per min to 250°C and then remained at 250°C. Hydrogen was used as the carrier gas at a flow rate of 30 ml/min and the effluent solutes were detected by flame ionization.

Interactions with instars of **Plutella**. In these tests, **Delia** adults were presented with 'clean' plants and plants on which either 5-10 or 30-40

Plutella eggs had been laid. In other experiments, the **Plutella** eggs were removed from the plants prior to testing. In subsequent experiments, flies were given a choice of 'clean' plants or plants infested with either 5 or 20 larval instars of **Plutella**. The higher density represents about 3-4 insects/leaf.

Interactions with **Brevicoryne** populations: 'Clean' plants and plants infested with either 30 or 120 aphids were tested for their effects on host plant selection by **Delia**. In other experiments, the aphids were removed from the plants immediately prior to testing.

Analysis of data: Data were compared by analysis of variance and the results expressed as least significant differences (L.S.D.) at $P = 0.05$.

3. Results

The effect of spraying suspensions of frass from the two **Pieris** species was similar to spraying the test plants with suspensions of macerated tissues from the leaves of their respective host-plants (Table 1). In contrast, fewer eggs were laid on plants sprayed with a frass suspension from **Plutella** larvae reared on both Chinese radish and horse-radish. Similarly, frass from **Phaedon** and **Mamestra** had no effect on **Delia** oviposition when the insects fed on four of the six plant types but reduced oviposition when the insects fed on the other two. On all six types of plant, **Evergestis** larvae produced frass that deterred oviposition by **Delia**.

Table 1. Oviposition by **Delia** on 'clean' cauliflower plants and plants sprayed with 4 ml of various frass suspensions. Data are expressed as, (+) treatment increased oviposition, (-) treatment deterred oviposition and (o) treatment had no significant ($P = 0.05$) effect.

Insect	Plants used to rear insects for production of frass					
	Cauliflower & cabbage	Swedes	Chinese radish	Horse-radish	Garlic mustard	Wallflower
(Macerated leaf)	+	+	o	o	-	o
P. brassicae } *P. rapae*	+	+	o	o	-	o
Plutella	+	+	-	-	-	o
Phaedon } *Mamestra*	o	o	-	-	o	o
Evergestis	-	-	-	-	-	-

Figure 1 shows that the volatile sulphur compounds characteristic of cauliflower plants do not pass through the insect unchanged, even with insects like **Pieris**. In all frass analysed, the isothiocyanates were

reduced almost completely to their respective nitriles.

Delia was also deterred from laying on plants on which **Plutella** has already laid (Table 2). In contrast, it prefered to may on plants infested with second- or fourth-instar **Plutella** larvae.

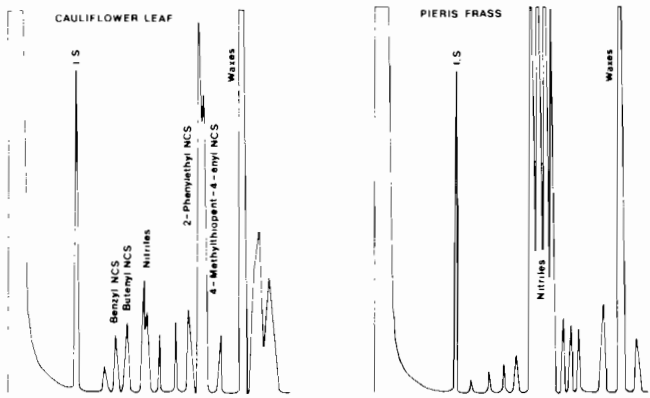

Fig. 1. Chromatograms of volatile isothiocyanates (NCS'S) and nitriles from cauliflower leaf and from the corresponding frass produced by **Pieris**. I.S. = Internal Standard.

Table 2. Number of **Delia** eggs laid on plants infested with 30-40 eggs or 20 larvae of **Plutella**. *Different from control (P = 0.05).

Stage of *Plutella* on infested plants		Mean No. of *Delia* eggs laid/plant		
		'Clean' plants	Infested plants	L.S.D.
Eggs	Present during test	97	56*	20
	Removed prior to test	99	59*	26
Larvae	First instar	69	63	32
	Second instar	84	125*	35
	Fourth instar	64	145*	31

Fewer **Delia** eggs were laid on plants infested with 120 **Brevicoryne** than on 'clean' plants (Table 3). This result was reversed when the aphids were brushed from the infested plants prior to testing.

Table 3. Numbers of **Delia** eggs laid on cauliflower plants infested with colonies of **Brevicoryne**. *Different from 'clean' control (P = 0.05).

Aphids/plant	Mean No. of *Delia* eggs laid/plant			
	0 ('clean')	30	120	L.S.D.
Aphids present during test	133	131	50*	29
Aphids removed prior to test	43	128*	140*	22

4. Discussion

Host-plant selection by **Delia** is influenced by the odour (Finch, 1978) and colour (Prokopy et al., 1983) of a wide-range of cruciferous plants. The present results showed that it is also influenced by other insects competing for the same resource. Although **Delia** females lay in the soil adjacent to plant stems, they first have to probe the surface of the plant to determine whether it is a suitable site for oviposition. The results in Table 1 show that, during this process, host-plant selection is influenced by the frass of other insects and also by the plant they are feeding on (cf. Renwick & Radke, 1985). Species such as **Pieris** advertised their presence to **Delia** in a positive way by producing larger amounts of chemicals similar to those released normally from damaged plants. Although such species reduced the sulphur compounds in their diet to nitriles in their frass, such changes did not deter **Delia** oviposition. In contrast, larvae of **Phaedon** and **Mamestra** seemed able to disguise their activity on four of the host plants by producing frass that did not influence host-plant selection by **Delia** (Table 1). This was not the case with **Evergestis** since all frass produced by **Evergestis** larvae deterred **Delia** oviposition, irrespective of host-plant. **Delia** oviposition was also deterred when **Plutella** had previously laid on the plants (Table 2), indicating that a marking pheromone from **Plutella** may have influenced **Delia** behaviour. Finally, **Delia** was deterred from laying on plants infested by the aphid **Brevicoryne** by the physical disturbance of the aphids or an alarm pheromone, since infested plants were more preferred when aphids were removed immediately prior to testing.

When the chemicals producing these interactions have been identified, they may show potential for use in integrated pest control systems. Before such chemicals can be used in practice, other than as attractants in insect traps, they will first have to undergo rigorous tests to ensure they are both relatively sage and environmentally acceptable.

Abstract

Host plant selection by **Delia radicum** is influenced considerably by other cruciferous-feeding insects competing for the same resource. Frass produced by larvae of **Pieris brassicae/rapae** and **Plutella xylostella** feeding on brassica plants stimulated **D. radicum** to lay whereas frass from **Phaedon cochleariae** and **Mamestra brassicae** had not effect and frass from **Evergestis forficalis** deterred **D. radicum** oviposition. **D. radicum** oviposition was also reduced around plants on which **P. xylostella** had laid and by the presence of colonies of the aphid **Brevicoryne brassicae.**

Key-words: oviposition stimulants, oviposition deterrents, **Brevicoryne brassicae, Delia radicum, Evergestis forficalis, Phaedon cochleariae, Pieris brassicae, Pieris rapae, Plutella xylostella, Mamestra brassicae.**

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INFLUENCE OF PHENOLGLUCOSIDES ON THE DISTRIBUTION OF HERBIVORES ON WILLOWS

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Variation in the biochemical characteristics of host plants and their importance as key factors in explaining the distribution of herbivores is a widely studied area (review in Rosenthal & Janzen, 1979). First we will review briefly some of the evidence showing how one group of plant secondary compounds, the phenolglucosides, present in the leaves of willows and poplars, could influence herbivore distribution, and more particularly the leaf beetles of the subfamily Chrysomelinae which are important among the herbivores causing leaf damage to willows.

According to Thieme (1965) and Palo (1984) phenols are the only group of secondary metabolites present in significant amounts in the leaves of the Salicaceae. However, they are not present in the leaves of all species of willow and poplar. The toxicity of these phenolglucosides is established (Vickery & Vickery, 1981).

In a recent study, Tahvanainen et al. (1985) showed that the concentration and the composition of the different phenolglucoside blends are species specific in willows. For 4 common leaf beetle species (**Phratora vitellinae, Plagiodera versicolora, Lochmaea caprea, Galerucella lineola**), The patterns of food plant selection observed in multiple choice preference experiments are closely related to the phenolglucoside spectra of the willows tested. Indeed, the second choice of the beetles was always the willow species which was chemically the most similar to the preferred host plant. The phenolglucoside blends can have both stimulatory and inhibitory influences on the leaf beetles.

The phenolglucosides of the leaves can also be used as precursors of the glandular defensive secretion of the larvae. Indeed, the secretion of salicylaldehyde by the larvae of several chrysomeline species feeding on salicaceous trees offers an excellent example of the use of plant secondary compounds in insect defense (review in Rowell-Rahier & Pasteels, 1986). Pasteels et al. (1983) have previously demonstrated the utilisation of the phenolglucoside salicin as the substrate for the production of salicylaldehyde. Since then, using the methods described by Rowell-Rahier & Pasteels (1982), we have investigated the role of salicortin, a further phenolglucoside found in the leaves of many willows, and have demonstrated that this compound, an acetylated salicin derivative, can also be used by the larvae of the beetle **Ph. vitellinae** as a precursor of salicylaldehyde.

Smiley et al. (1985) recently studied the relationship between a Californian Sierra Nevada beetle (**Chrysomela aenicollis**) and its salicaceous host plants. The willow leaves they encounter in the field have

highly variable concentrations of salicin, salicortin and tremulacin. No other phenolglucosides could be detected in significant amounts. In leaves of **S. lasiolepis** the salicin concentration varied between neighbouring plants by one hundred fold (from 0.08 to 8% DW). Damage due to herbivory is significantly higher on plant specimens rich in salicin and beetle larvae placed on high salicin plants have a higher survival rate.

The importance of phenolglucosides as defensive precursors for some chrysomelines thus established, we decided to compare the influence of phenolglucosides (mainly salicin and salicortin) on the distribution in the field of herbivores using and not using these compounds for their own benefit. This is the subject of this paper.

An ideal site for this study was offered by a plantation (Gramont, Belgium) of small willow trees which included both **S. alba** and **S. fragilis** together with numerous hybrids between these species. **S. alba** contains very little or no phenolglucosides in its leaves whereas **S. fragilis** is rich in phenolglucosides. 89 individual trees were randomly selected. A sample of the leaves of each to them was collected and analysed for phenolglycoside content. All herbivores present on these trees were counted; this was possible only because of the small size of the young trees. Variation in phenolglucosides and in insect distribution within individual trees was not considered in this study. Detailed data are presented in Soetens (1986). We will discuss only the results which concern the 3 main herbivore species encountered. These are:

Phratora vitellinae, a Chrysomeline, the larval of which use phenolglycosides for their defense;

Plagiodera versicolora, a Chrysomeline, the larval defense of which is independent of host plant secondary chemistry, and

Pontania proxima, a gall forming sawfly, which was the most numerous herbivore encountered.

The adults of both leaf beetle species have a low motility.

Before discussing the results, we would like to make what we believe to be a very important methodological comment. To analyse the phenolglucoside content of the leaves we first used a time-consuming methanol extraction (3 consecutive extractions in a large volume of solvent) followed by reverse phase HPLC (methods in Meier et al., 1985). To spare time, we then tried to replace this precise method by a quicker and simpler water extraction (overnight in a small volume of water) followed by semiquantitative TLC (methods in Smiley et al., 1985). We of course ran controls to compare all 4 aspects of the 2 procedures. That is, we compared water and methanol extracts of the same material, and we also compared TLC and HPLC analysis of the same extracts. It became clear that the analysis methods (TLC and HPLC) gave comparable results, but that the extraction methods (large volume of methanol or small volume of water) gave very different results. Moreover, when we analysed the data, there was nearly no correlation to be found between herbivore distribution and the salicin and salicortin content of methanolic leaf extracts. Relatively clear correlations could however be observed between herbivore distribution and glucoside content determined phenol from aqueous leaf extracts. This, in fact, is perhaps not surprising, because after all the digestive systems of insects are not full of methanol.

In Table 1, the results of a multiple regression analysis are summarised. The dependent variables are the abundance of each of the herbivores studied and the independent variables are the content in 4 different phenolglucosides of aqueous leaf extracts. Because the phenolglucosides might influence the defense of larvae but not that of adults, the adults and the larvae of **Ph. vitellinae** and **Pl. versicolora** were recorded separately.

The variation in the content of phenolglucosides has no clear impact on the distribution of **Ph. vitellinae** adults. We have shown in previous studies that at least salicin is not a phagostimulant for this species at the concentration present in the leaves, but rather that physical factors, such as leaf trichomes, are important in determining food plant choice. Trichomes are commoner on leaves of species poor in phenolglucosides.

The larvae of **Ph. vitellinae** show a weak correlation with the phenolglucoside content of the leaves. We expected this result because the plant phenolglucosides are important as larval defensive precursors for this species. The most important component of the multiple correlation is the content in P3, a still unidentified compound.

Table 1. Multiple regression analysis.

Independent variables: salicin, salicortin (=SC), P3, P4.

	% VARIATION EXPLAINED	OVERALL SIGNIFICANCE	MAIN@ COMPONENT
Dependent variable			
Ph. vitellinae			
Adults	4	ns	SC
larvae	8	*	P3
Pl. versicolor			
Adults	24	***	SC
Larvae	1	ns	-
P. proxima			
Galls	30	***	P3 SC

ns, not significant; *, p<.10; ***, p<.005.
@, single independent variable with the largest influence.

The pattern for **Pl. versicolora** is somewhat different. The distribution of the adults of this species is highly positively correlated with the phenolglucosides content of the leaves. A stepwise multiple regression showed that only one of them, salicortin, was responsible for this: 22% of the variation in abundance of **Pl. versicolora** adults can be explained by the variation in salicortin content of the leaves.

The distribution of the larvae, on the other hand, does not seem to be influenced at all by the variation in phenolglucosides, and indeed this species does not use them as precursors for its larval defense (Rowell-Rahier & Pasteels, 1986).

The most numerous herbivore species in the studies willow field was the gall-building sawfly **Pontania proxima**. These are also highly positively correlated with the phenolglucoside content of the leaves. 30% of the variation in gall number can be explained by variation in

phenolglucoside content. Among the 4 compounds analysed, P3 and salicortin have the largest influence. It is possible that the sawflies select plants which are rich in phenolglucosides so that their larvae benefit from the extra protection brought by the larger quantity of phenolglucosides in the gall tissue. This hypothesis remains to be examined.

Thus, the distribution of the three most frequent herbivores on the studied willows can in part be predicted by the phenolglucoside content of the leaves. A further point remains to be examined: are there any interactions between the three species?

To answer this we did some further multiple regression analyses looking at the relationship between the distribution of each species, for example **Plagiodera versicolora** adults (see Table 2), and both the phenolglucosides and the abundance of the other herbivores on the tree. It is clear from this analysis that the abundance of **Plagiodera** is positively correlated with the phenolglucosides (among them salicortin plays the largest role) and the abundance of **Ph. vitellinae** larvae and the galls of **Pontania proxima**. 47% of the variation in the number of **Plagiodera** adults can be explained by this set of independent variables. This means that at least on the level of the individual tree the 3 species do not exclude each other. On the contrary, all 3 of them favour the same individual trees rich in salicortin. The phenolglucosides alone explained only 24% of the variation. This observed distribution must be explained by other factors, for example, some plant characteristics which have a positive influence on the 3 species and are positively correlated with the salicortin content of the leaves. Also, we can not exclude the possibility that the glucosides could be an induced defense response of the tree to herbivory.

Table 2. Multiple regression analysis.

Dependent variable: Pl. versicolora adults
Independent variables: salicin, salicortin, P3, P4, Ph. vitellinae, adults, Ph. vitellinae, larvae, P. proxima.

Multiple correlation coefficient: .68
variation explained: 47%
Overall significance: p<.001

PARTIAL COEFF. FOR:	BETA	P	
Salicin	.013	.87	ns
Salicortin	.221	.12	ns
P3	.024	.84	ns
P4	-.035	.73	ns
Ph. vit. adults	.030	.73	ns
" " larvae	.301	<.001	***
Pont. prox. galls	.332	<.001	***

Our results suggested to us that the effects of the glucosides on the distribution of the beetles were different for the 2 species observed. To explore the cause of these difference, we are planning to test directly the phagostimulant or deterrent effect of salicin and salicortin in the laboratory by giving the beetles the choice between extracts of a control plant (without phenolglucosides) to which increasing quantities of salicin

and salicortin are added. To explain the larval distribution, we are planning to test the impact of salicin and salicortin on oviposition. Preliminary results of John Smiley (pers. comm.) suggest that for **Ch. aenicollis**, which use glucosides as larval defensive precursor, salicin stimulates oviposition.

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PYRAZINES AS ALERTING SIGNALS IN TOXIC PLANTS AND INSECTS

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A feature of alerting signals is that they cut across the man-made biological classifications and the same token may be used by animals or plants, vertebrates or invertebrates, ranging from continent to continent and ocean to ocean.

For those of us who possess colour vision, red (and orange/red) is the most obvious example of an alerting signal. It warns birds if potential prey is dangerous and attracts them to edible fruit and berries. A variety of red flowers draw their avian pollinators to a supply of delectable nectar, while baboons allure their mates with bright red, swollen posteriors. Birds, frogs, fish, lizards, snakes, spiders, crustacea, molluscs, beetles, butterflies and plants all use red to warn predators of their toxic or dangerous qualities or to threaten or intimidate rivals. Turkeys use their red wattles to indicate both love and hate.

Clearly blood can function as a warning signal. In the snowy Antarctic wastes it is a strikingly conspicuous feature round seal holes where a kill of some unwary penguin has occurred, and the fainting Victorian heroine, so beloved by 19th century novelists, is a romanticised example of the same phenomenon. Today we ourselves use red for traffic signals, letter-boxes, dangerous sleeping pills, children's candies, Father Christmas's festive attire and the uniform of the Life Guards. Finally we have perfected a crimson rose, the Etoile d'Hollande, to excite and convey our love and desire.

The evolution of red as the alerting signal par excellence must have been due, chiefly, to the dire need for a contrasting colour in a green world beneath a blue vault. But it serves the same purpose in the desert. One wonders what destined this colour to become a danger signal which spans the Phyla and geographical barriers and defies time and change, so that the message has come down the ages unambiguously in every continent. It is worth noting that red shows a high degree of saturation and is a very pure colour. Furthermore the red/green cone system forms about eighty per cent of the total colour receptor system in our eyes. It is also suggested by one of us (Barry Moore) that the signallers would have adopted the low energy region of the spectrum, since it is relatively easy to make an organic pigment that absorbs the high-energy violet and blue (to appear yellow and orange) or violet to green (to appear red) but the molecular requirements for absorbing the low energy region are much more demanding.

Did the message ante-date the vertebrate eye and even flowering plants? Were the giant carboniferous dragon-flies endowed with colour

vision? We suspect they were. Could the original red signals, based perhaps on sequestered carotenoids, have been invented by insects for insects? A concentration of carotenoids - of which 100 million tons are produced annually by plants today (Borenstein & Bunnell, 1966) - sequestered from vegetation, seems the most likely source of the first red alerting signal.

Let us now turn to alerting odours. We do not know why - but in a lecture, a switch from the subject of colours to smells seems to engender a burst of spontaneous laughter - unless this is due to the truism that the Lord is a super chemist but a rather indifferent plumber.

Surprisingly little is known about alerting odours, possibly because we ourselves are so poorly endowed, and in comparison with, say, a dog or an antelope, have a lamentably deficient sense of smell. We can obtain some idea of the power of odour-memory from dogs. Time and fashion can so alter a person's appearance that we fail to recognise them. But dogs never forget friend or foe - a transvestite can't fool the poodle.

Entomologists have long since recognised that many flowers and butterflies share an alerting scent - a sweet etherial perfumed vanilla and faint chocolatey smell. This we have assumed (Rothschild, 1964) is a means by which plants attract nectar-hungry potential pollinators to their flowers while butterflies draw other butterflies to the area. The mutually beneficial alerting perfume thus generated favours mating opportunity for the insects, and may also advertise the presence of precursors of sex pheromones such as pyrrolizidine alcaloids, and simultaneously improve fertilization of the plants.

The best example of an alerting scent is that produced by the pyrazines, six-numbered aromatic rings with two nitrogen atoms placed opposite each other. Over 100 pyrazine compounds have been identified but those we found functioning most consistently as part of warning systems are the 2,5-dimethyl,3-alkyl- and 2-methoxy, 3-alkyl-pyrazines, which produce one of the most powerful odours known to man (Moore and Brown, 1981; Rothschild et al., 1984).

We have identified those compounds in various unrelated but well-defended plants such as Poppies (**Papaver**), Ragwort (**Senecio jacobea**), Milkweeds (**Asclepias**), Stinging Nettle (**Urtica dioica**), and Milk Thistle (**Silybum**), and equally varied well defended aposematic insects such as the Monarch Butterfly (**Danaus plexippus**), Tiger Moths (**Arctiids**), Burnet Moths (**Zygaena**), Syntomid Moths (**Amata**), Ladybirds (**Coccinella**), Lycid beetles (**Metriorrhynchus**) and one of its Cerambycid mimics, as well as in the mandibular glands and poison glands of certain ants (implicated in trail laying (Attygalle & Morgan, 1985)). We have also detected them by sniffing in certain aposematic frog-hoppers (Rothschild, 1961), a grasshopper and a large array of 'protected' species not so far tested by us.

In some cases such as the Cinnabar Moth (**Tyrea jacobaeae**) the insect manufactures pyrazines itself, but those of the Monarch Butterfly are sequestered from the food plant, and it lacks these compounds if reared on a plant free of pyrazines. A similar situation is found in the case of certain warning colours: many pigments are synthesized by the insect but others are plant-derived. In the case of Saturnid larvae, for example, the bright blue tubercles are coloured by pterobilins (secreted bile pigments)

and the yellow or red tubercles by sequestered carotenoids.

More subtle relationships exist with plants, which are not really understood. The nettle in flower, especially after rain, emits a strong smell of pyrazines. Ladybirds are found in some numbers sitting on the flowers. What are they doing there? Are they attracted to Aphids or to pollen or to the scent of the wet plants? In any case they add to the aposematic assembly and increase the level of warning signals. Along with them is a pretty, bright green, metallic weevil (**Phyllobius urtica**) which feeds on nettle and probably stores pyrazines, for it can well be described as iridescent and thus qualify as an aposematic species - or at least a dual signaller. Then we have a plethora of Nymphalid larvae consuming the nettle, all of which should profit from the disagreeable attributes of the plant and may possibly be sequesterers of pyrazine, if only at the larval stage. These aposematic assemblages on toxic plants involving both scent and colour, are extremely interesting. For one thing they make kin selection an unnecessary prerequisite for the evolution of warning qualities. Any specimen say, feeding on nettle or **Asclepias** and mutating towards bright or conspicuous colour, will find the necessary protective **entourage** in the form of the unrelated but aposematic individuals already present. Furthermore they will have the nettle's pyrazine umbrella to shelter them, quite apart from its other unpleasant characteristics.

Although the odour of pyrazines is unmistakeable, no two defensive secretions ever smell exactly alike. This is probably due to the fact that they are present in different concentrations, and also because pyrazines seem to modify other scents and flavours with which they come into contact, and when manufactured by the insect themselves are usually in the presence of a large number of chemicals in the poison glands and haemolymph, some of which are also odoriferous.

Pyrazines share with vanillin an interesting but poorly studied elusive characteristic: their smell is not only persistent but highly evocative. When first exposed to a whiff of pyrazine one stuggles to identify the odour, bewildered by its reminiscent quality and reminded of 'something' which, maddeningly seems to elude one, yet remains tantalizingly balanced on the knife-edge of remembrance. It is probably important for an alerting scent not so much to be intrinsically disagreeable but to remind the sniffer - to evoke - certain past experiences clearly, be they nice or nasty.

The most famous passage in world literature which deals with evocative aromas and taste is the description by Marcel Proust of the flood of childhood memories which overwhelmed him when he put the famous madeleine to this lips. As one of us has said elsewhere (Rothschild, 1986) it is unlikely that anyone has bothered to pass the aroma of that delectable little cake onto a gas chromatogram, but those of us who are involved with insect/plant relationship can guess that the evocative substance was vanillin, that orchid-derived flavouring of the classical French madeleine. We are left wondering how this evocative element works with bird and mammal predators, especially where toxic prey is concerned. One suspects it does not merely function as a powerful cue, warning the enemy of the plethora of protective devices stockpiled by aposematic plants and insects, but that in

some subtle way it sharpens memory and even improves learning.

Guildford (in prep) has carried out preliminary feeding experiments with purified pyrazines, supplied by one of us (Barry Moore) which have shown that chicks learn to avoid water laced with quinine and pyrazine, faster than water with quinine only, despite the fact that quinine alone is more aversive than pyrazine alone. Probably mammals will react more decisively than birds to the evocative quality of alerting scents, since unlike avian predators they depend on olfaction rather than sight for locating and identifying prey.

Pyrazines are in many ways ideal substances to function as alerting signals. They are readily available in miniscule amounts in non-toxic plants such as potatoes and peas, where they are responsible for 'interesting' harmless flavours but, when concentrated, produce a memorable stench. They can thus be sequestered and stored by an insect and, should the life-style demand it, "jacked up" to provide a warning odour.

The pyrazine nucleus is a stable one that is readily derived bio-synthetically from amino-acids and sugar degredation products. Hence, pyrazines are widespread not only in natural bouquets and flavours, but in the decomposition or pyrolysis products of biological tissues and by fungal decay. Evidently they have served as natural points of convergence in the evolution of various warning systems.

The possibility that the smell of pyrazines occurs in the smoke of forest and prairie fires is suggested by Professor Gunnar Bergstrom. In this case they could provide an almost universal terrestrial alerting signal - a sort of 'blue print' for all classes of aposematic animals and plants. Apart from a role unifying the possible lines of parallel evolution of pyrazine defensive scents in both plants and insects, forest and prairie fires would link sight and smell in the same warning system. The orange and scarlet colour of the flames would re-enforce the significance of the widespread use of red in visual aposematic effects.

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PLANT PRODUCED ALLELOCHEMICS AND THEIR INVOLVEMENT IN THE HOST SELECTION BEHAVIOR OF PARASITOIDS

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1. Introduction

Plants produce numerous chemical compounds. Some of these compounds influence the behavior of organisms, including insects, that feed on the plants. Also, insects attacking plant feeders are often influenced by allelochemics eminating from the plants (Nordlund et al., in press). In the following few pages I will discuss the involvement of plant produced allelochemics in host-habitat location and how the diet of phytophagous insects can influence host location by parasitoids. These interactions have important implications for the evolutionary history of the organisms involved and the use of entomophagous insects in applied biological control programs.

2. Host-habitat location by parasitoids

Host-habitat location is the first step in the process leading to successful parasitization. This step often places a greater limitation on the number of host species actually attacked by a parasitoid than does host suitability (Picard & Rabaud, 1914; Townes, 1960; Flanders, 1962). Many parasitoids exhibit a high degree of specificity in their choice of habitats in which to search for hosts. Vet et al. (1984), for example, reported that **Asobara tabida**, a parasitoid of Drosophilidae, prefer decaying fruit, where yeast is the predominant agent of decay. **Asobara rufescens**, on the other hand, prefer to search in decaying plant leaves, where bacteria are the predominant agent of decay. The specificity was so strong that **Drosophila** larvae in a fruit-yeast mixture, placed in a beet field, were parasitized only by **A. tabida** while **A. rufescens** females parasitized only larvae found in the adjacent decaying beet leaves.

In nature, a parasitoid may encounter several habitats and must locate the habitat most likely to contain suitable hosts. A number of different stimuli may be involved in host-habitat location, inducing plant shape, plant height, plant color, the amount of sunlight present, and allelochemics. A plant released allelochemic that increases the probability of a parasitoid attacking a herbivore feeding on the plant, is a synomone (Nordlund & Lewis, 1967). We are just beginning serious study of the roles synomones play in host-habitat location by parasitoids.

While conducting a study of insects in a corn-bean-tomato polyculture, Nordlund et al. (1984) found that parasitization of **Heliothis zea** eggs, by **Trichogramma spp.** was higher (42.9%) in plots of tomato than in adjacent plots of corn (1.5%), indicating that the **Trichogramma spp.** preferred to

search on tomato plants. Thus, hexane extracts of corn and tomato leaves were tested, using a petri dish bioassay and **T. pretiosum**, to determine if chemical stimuli were involved. Parasitization was significantly higher in petri dishes treated with the tomato extract (53.%) than in dishes treated with corn extract (30.4%) or hexane (36.1%) (Nordlund et al., 1985a).

A jar olfactometer was used to determine if a volatile synomone from tomato plants stimulates searching behavior (Nordlund et al., 1985b). In this experiment the females were exposed only to air that had passed through a glass tube treated with either a hexane extract of tomato leaves or with hexane. Parasitization was significantly higher in jars treated with tomato extract (20.5%) than in jars treated with hexane (9.2%), so tomato contains a volatile synomone that stimulates search behavior in female **T. pretiosum**. Similarly, the mean number of **T. pretiosum** females (5) found in the arm of a Y-tube olfactometer treated with tomato extract, after only 10 min, was significantly greater than the mean number (2) found in the control arm. This suggests that female **T. pretiosum** are also attracted by a synomone in tomato plants (Nordlund et al., 1985b).

In the same study, a field experiment was conducted on "Silver Queen" corn. In the treated plots, cotton rolls treated with tomato extract were suspended at mid-plant height by a thread. In control plots, cotton rolls treated with hexane were suspended in a similar manner. **H. zea** eggs were applied to simulated oviposition sites and **T. pretiosum** were released in the plots. **H. zea** eggs were applied and collected for 3 readings. The results of this experiment show that tomato extract increased parasitization in all three readings even though direct contact with the material was negligible (Table 1).

Table 1. Parasitization of **Heliothis zea** eggs by **Trichogramma pretiosum** in plots of "Silver Queen" corn in which cotton rolls, treated with a hexane extract of "Floridade" tomato leaves or hexane, were suspended. [1]

Reading	Exposure (hr)	N	Mean Percentage of Parasitization In Extract Plots	Hexane Plot
1	4	30	49.5a	28.8b
2	18	28[2]	44.7a	19.7b
3	4	20[3]	30.3a	16.4b

1. Means for each reading followed by different letters are significantly different as determined by ANOVA. (Data from Nordlund et al., 1985b).
2. In 2 treated plots no eggs were recovered so these replications were eliminated from the analysis.
3. The 3rd reading of one test was destroyed by rain.

The studies discussed above clearly demonstrate that tomato plants contain a synomone that influence the host-habitat location behavior of **T. pretiosum** females.

3. Effects of host diet on host location by parasitoids

Sequestration of chemicals from food sources that serve as

allelochemics has been observed in some insects species. For example, larvae of the sawfly, **Neodiprion sertifer**, sequester the terpenoid resin of its host plant (**Pinus sylvestris**) and use this resin in defense (Eisner et al., 1974). Kairomones that play an important role in the host location behavior of parasitoids may also be sequestered from the food plant of the host. The Tachinid parasitoid **Lixophaga diatrea** is stimulated to larviposit by a kairomone found in the frass of its host **Diatraea saccharalis**. This chemical, or its precursor, is sequestered from sugarcane, and larviposition is not stimulated by frass from larvae fed on a soybean flour-wheat germ diet (Roth et al., 1982). Sauls et al. (1979) demonstrated that the diet of **H. zea** larvae affected the kairomonal activity of larval frass for the parasitoid **Microplitis croceipes**. Homogenate of frass from larvae reared on Pink Eye Purple Hull Cowpea cotyledons received a significantly higher response (2.6) than did a homoginate of frass from a laboratory diet (0.3). There was no response to homoginates of cowpea cotyledons or laboratory diet. In a later experiment, homoginates of frass from H. **zea** larvae fed on soybean, cotton, or corn fruiting bodies were compared to a homoginate of frass from laboratory diet-fed larvae (Nordlund & Sauls, 1981). The data (Table 2) show that larvae fed on corn produce frass with no stimulatory effect on **M. croceipes** females. Interestingly, though **H. zea** is an important pest of corn, **M. croceipes** females are not found in corn fields (Smith et al., 1976), thus **H- zea** feeding in corn escape parasitization by this species and build up substantial populations in corn fields.

Table 2. Response of **Microplitis croceipes** females to homoginates of frass from **Heliothis zea** larvae on either corn, cotton, soybean or lab diet.[1]

SOYBEAN	COTTON	CORN	DIET
1.6a	1.0b	0.0c	0.3c

1. Means followed by different letters are significantly different as determined by Duncan's New Multiple Range Test. (Data from Nordlund & Sauls, 1981).

Microplitis demolitor is another larval parasitoid found to respond to a kairomone(s) in the frass of noctuid larvae. Nordlund and Lewis (1985) reported that diet affects the response of this parasitoid to frass (Table 3). Parasitoids responded to a hexane extract of the frass, which demonstrates that the response is elicited by an allelochemic.

4. Conclusion

A great deal of the literature on chemically mediated insect-plant relationships involves kairomones that attract phytophagous insects and stimulate their feeding or allomones that offert plants some defense against insect attack. Interactions between plants and insects of the third trophic level remain relatively unstudied, despite a number of references to the importance of these interactions. Numerous examples of such interactions can be found in the literature (Nordlund et al., in press).

The examples presented in the previous pages are by no means unique.

We have seen that synomones in tomato plants stimulate behavior that results in increased rates of parasitization of **H. zea** eggs by **T. pretiosum** in both the laboratory and field and that corn, another plant attacked by **H. zea**, does not possess such stimuli. This explains why parasitization rates were much lower in plots of corn than in adjacent plots of tomato. Application of appropriate synomones to corn resulted in increased rates of parasitization. Slow release formulations of such synomones may be quite useful in biological control programs. We have also seen that diet can effect the chemical nature of the host and thus the response of parasitoids to host insects.

Table 3. Response of **Microplitis demolitor** females to frass of **Heliothis zea** and **Trichoplusia ni** larvae reared on CSM laboratory diet or Pink Eye Purple Hull Cowpea cotyledons, and to frass extract.[1]

Treatment	No. of females responding on pass 1	2	3	No. females exhibiting no response
	H. zea larval frass[2]			
CMS Diet	1	0	1	28
Pea cotyledons	21	2	0	7
	T. ni larval frass[3]			
CMS diet	0	0	0	30
Pea cotyledons	16	1	0	13
Hexane extract of frass from *H. zea* larvae fed on pea cotyledonss[4]				
Hexane	0	0	0	30
Frass extract	19	0	0	11

1. Data from Nordlund & Lewis, 1985.
2. df=3, X2=33.782, Prob.=0.0001
3. df=2, X2=23.721, Prob.=0.0001
4. df=1, X2=27.805, Prob.=0.0001

We are just beginning to understand the importance of interactions between plants and entomophagous insects. Entomophagous insects can cause heavy mortality in phytophagous insect populations and are a cornerstone of applied biological control of insect pests. A great deal of research is being directed at the development of techniques for utilization of entomophagous insects for pest control. Thus the types of interactions discussed above are potentially of great practical importance. These interactions are also of extreme interest academically.

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THE INFLUENCE OF VOLATILE PLANT ALLELOCHEMICS ON THE THIRD TROPHIC LEVEL (PARASITOIDS) AND THEIR HERBIVOROUS HOSTS

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1. Introduction

Insect-plant relationships not only include interactions between plants and herbivores or pollinators, but also with a third trophic level, parasites, parasitoids and predators of plant herbivores. From a plants point of reference, both the pollinators and members of the third trophic level can be of benefit to the plant. The ability of plants to manipulate pollinators is well documented (Kevan & Baker, 1983; Dafri, 1984; Yeo, 1972). In contrast, the ability of plants to manipulate the third trophic level is represented by a few scattered reports (Janzen, 1966; Read et al., 1970) and by an abundance of anecdotal information (Vinson, 1981). However, as Price stated (Price et al., 1980; Price, 1981) ecological models based on only two trophic levels (Rhoades & Cates, 1976; Rosenthal & Janzen, 1979; Lawton & McNeill, 1979) provide important concepts by fall short of broad ecological theories.

Plants may manipulate the different trophic levels in many ways, but the role of volatile allelochemicals in attracting or repelling herbivores and parasitoids, and the effect of toxic allelochemicals on herbivores, both directly and acting through the herbivore on parasitoids, are of particular interest. Plant volatile chemicals can play many roles. They may provide an insect with information regarding the location of a plant as a source of food or shelter or as an object to be avoided, or as starting point for a search for food or a mate, or a reproductive resource which might be associated with the plant.

2. Procedures

We began our studies utilizing cotton, **Gossypium hirsutum** as the plant; the tobacco budworm, **Heliothis virescens**, as the herbivore; and the ichneumonid, **Campoletis sonorensis**, as the parasitoid. Using a Petri dish bioassay, a "Y"-tube olfactometer, and a filtered air flight chamber olfactometer, we have examined the role of plant volatiles influencing the behavior of **C. sonorensis.**

The attractive compounds were determined by extracting plant parts in diethyl ether, reducing by vacuum to near dryness at 27°C, chromatographing over florisil (60/100A) using EtOAc-hexane-HOAc (10:90:0.25) as a developing solvent, and separating and collecting the organic compounds by gas chromatography using a 1.83 mm x 4 mm -id glass column of 5% OV-101 on chromosorb G, 80-100 mesh, He flow of 60 ml/min, operated isothermally at 160°C. Individual peaks were collected in 40-cm x 1-mm-id glass capillary

tubes with liquid nitrogen cooling, and were rechromatographed on 5% OV-275 on Chromosorb G, 80-100 mesh. Following confirmation of the biological activity of purified GC fractions, the samples were subjected to mass-spectral and nuclear magnetic resonance spectroscopy and their structure ascertained. The chemical structures was confirmed by comparison to known compounds or through synthesis along with confirmation of the biological activity through bioassays.

To investigate the effects of various terpenes isolated from cotton on the growth and development of **Heliothis**, the chemicals were applied to alphacel, the solvent removed and the alphacel added to artificial diet. First instar larvae were placed on the treated diet and their weights were periodically determined and compared to controls.

3. Results

3.1. The plants role in attracting the third trophic level

Plant such as cotton, tobacco, and sorghum were found to be significantly more attractive to **C. sonorensis** than chickweed or wild carrot, the former being known hosts of **Heliothis** (Elzen et al., 1983). It was also observed that the plant terminals and buds were more attractive than stems, while roots elicited only a very weak response. Since young **Heliothis** larvae are often located on cotton terminals (Pieters & Sterling, 1974), the increased attractiveness of the terminal region is at the very least fortuitous.

Diethyl ether extracts of cotton plant tissue (var. Stoneville 213) consistently elicited a strong attraction from **C. sonorensis**. Purification and identification of the active fractions of the diethyl ether extracts by column and gas chromatography yielded five monoterpenes (myrcene*,α-pinene, β-pinene, limonene and trans-ocimene*) and eight sesquiterpenes (copaene*, β-caryophyllene,α-humulene*,γ-bisabolene*,β-caryophyllene oxide*, spathulenol*, β-bisabolol*, and gossonorol*). Of these terpenes two of the monoterpenes and six sesquiterpenes (starred) were attractive to **C. sonorensis** (Elzen et al., 1984a).

We also examined the volatile profile of many cultivars of cotton. Several distinct differences were found and the glandless cultivars had little or no volatile terpenes (Elzen et al., 1986). Glands found in cotton plant tissue were known to be storage vessels for non-volatile terpenoid aldehydes such as gossypol (Stanford & Viehoover, 1981) which are thought to be involved in increasing the plants resistance to herbivores. Glandless varieties are known to have little resistance to **Heliothis** attack. Due to these findings, we collected the oil from glands of the cotton variety "Acala" and compared the concentration of volatiles of this oil to that of the oils and fluid in the surrounding tissues. We founds that the gland oil contained 31,000 ppm of total volatile terpenes while a 100-fold volume of surrounding tissue fluid contained no detectable terpenes (Elzen et al., 1985), indicating that cotton volatile terpenes are confined principally to the subepidermal glands. While both the terpenoids aldehydes such as gossypol, hemigossypol, and the heliocides, as well as the mono and sesquiterpenes such as gossonorol, bisabolol, and myrcene are found in the glands, the ratio of terpenoid aldehydes to the mono and sesquiterpenes

differed greatly between the various cultivars. Thus the question became "do different cultivars differ in their attractiveness to parasitoids and do different cultivars differ in their effect on the quality of the host as a resource for parasitoid development"'. This is particularly important since several researchers have shown that secondary plant compounds, such as tomatine and nicotine (Campbell & Duffey, 1979; Barbosa & Saunders, 1984), can result in reduced parasitoid quality presumably due to the reduced quality of the herbivore to serve as a host (Vinson & Barbosa, in press).

Our preliminary study of the flight behavior of **C. sonorensis** to glanded and glandless cultivars showed that the parasitoids responded less frequently to the glandless variety (Williams et al., in press). Further experiments have shown that C. **sonorensis** responds significantly greater proportion to glanded cultivars than to glandless cultivars or diploid cotton (Elzen et al., submitted for publication). These results point out the importance of the volatile chemicals in the orientation of **C. sonorensis** to plants and suggest that plant breeding can reduce or increase the attractiveness of plants to the third trophic level.

Since plants were found to be attractive to **C. sonorensis**, we next compared the attractiveness of cotton against **Heliothis**, cotton infested with **Heliothis** and a blank control in a four choice bioassay. Results demonstrated that cotton infested with **Heliothis** was more attractive than cotton alone which was more attractive than **Heliothis** alone (Williams et al., in press). We also found in an ovipositional preference bioassay that **Heliothis** which were fed cotton were preferred over **Heliothis** fed an artificial diet. Chemical analysis of larvae fed both diets revealed the presence of cotton volatiles only in the cotton fed larvae, the volatiles being primarily in the frass of **Heliothis**. **Campoletis sonorensis** responded significantly more often to the frass of cotton fed larvae indicating that the volatiles could influence both host location and host acceptance (Elzen et al., 1984b). We also found that **C. sonorensis** responded more often to damaged than to undamaged cotton leaves.

3.2. The plant's role in affecting the quality of the herbivore as an ovipositional resource of the third trophic level

The results of these studies indicate that parasitoids can be attracted by the food plant that harbors their hosts and volatiles in the plant directly or indirectly through the host can influence the parasitoid's ability or locate the host. However, such herbivores may not be suitable for the development of the parasitoids progeny. As discussed by Vinson and Barbosa (in press), once a host is located and an egg placed in or on it, the host represents a container supplying the needs of the developing parasitoid within the constraints that the host represents. Allelochemics produced by the food plant of the host may reduce the suitability of the herbivore (host) for parasitoid growth and development.

The fact that certain plant compounds or the plant's nutritional quality can effect the quality of a host as a resource for a parasitoid has been documented (Campbell & Duffey, 1979; Barbosa & Saunders, 1984; Greenblatt & Barbosa, 1981). In our studies of the effect of host plant

chemistry on parasitoids, we began with chemicals which are believed responsible for resistance to herbivore damage (Williams et al., in press). Feeding studies incorporating gossypol had shown that larval development of **Heliothis** was retarded (Chan et al., 1978). We found that small dosages of gossypol stimulated growth while larger dosages retarded growth as expected (Stipanovic et al., in press). When **H. virescens** larvae were fed one week on a diet containing a gossypol dosage calculated to reduce larval growth by 50% and then served as a host for the parasitoid **C. sonorensis**, the emergence time and percent emergence of the parasitoid did not differ, but the average weights of the adults were reduced.

We have also shown that some of the volatile terpenoids, which are also found in the subepidermal pigment glands along with gossypol (Stanford & Viehoover, 1981), can affect the growth of larval **Heliothis.** Caryophyllene oxide retards the growth of **Heliothis** at high dosages and acts synergistically with gossypol at both high and low dosages, (Williams et al., in press). Caryophyllene has a small growth retarding effect at low concentrations which does not change at higher dosages (Williams et al., in press). The levels of the volatile and other cotton terpenoids vary among cotton cultivars (Elzen et al., 1986; Yang & davis 1976) and can be altered through selective breeding. How such mixtures affect the quality of the herbivore to serve as a host for the parasitoid is yet unknown, but the preliminary results suggest such mixtures could have a major impact on the interactions among the three trophic levels.

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CHAPTER 3. OLFACTION, VISION, GUSTATION, AND TACTILE SENSATIONS IN PLANT INSECT RELATIONS

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PLANT RECOGNITION BY INSECTS: A CHALLENGE FOR NEURO-ETHOLOGICAL RESEARCH

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Studies of ecosystems explain the shifting of energy (matter) from plants to animals and through the soil and/or air back to the plants but usually pay little attention to the essential role which the animal senses play in such cascades of events (see Remmert, 1984, p. 85, who speaks of "ecological neurobiology" in this context). Obviously, the plant-consuming animals are selective and their sensory capacities need to be considered. These more or less specialised sensory systems probably developed by coevolution with the respective defensive properties of the plants. Switching and chanelling of the gross energy fluxes is thus critically depending upon the properties of the plants and the corresponding animal senses. It is important to realize in this context that the transfer of energy is negligibly in these "recognition processes", since sensors only "take" minute probes of the energy of the environment. When considering the expenditure of energy in mutual plant-insects adaptations, however, one needs to remember that the production of defensive plant structures and substances is costly. These products are not just minute traces of matter. Furthermore, the neural apparatus which insects developed to find and recognize plants is, in principle, costly too, although these structures are also be used for other purposes.

The process of recognition of a plant or part of it by an animal is clearly what psychologists call "**Gestalt Recognition**" which means sensory selections of certain essential features of a person or a thing. To accomplish the Gestalt Recognition of a plant, several more or less interacting sensors have to probe the plant on the basis of its form, colour, surface structures and chemistry. In the terminology of the ethologists, the behaviourally relevant gestalt of a plant (the search image of key stimulus) would be the innate or learned factor which gives rise to a corresponding behaviour. How precise, how sharp is then this image in reality? The answer is difficult because of three major variables which come into play. The first and second relate to the "sender", the plants, and the third to the "receiver", the insect: 1) phylogenetic neighbourhood of plants (closely related plants look, feel, smell and taste more similar than less closely related ones); 2) ecological adaptations (such as structural similarity of non-related plants such as succulents and vines); 3) herbivore adaptations (monophages rely on "sharp" plant images while polyphages are satisfied with "blurred" images).

After these very general comments, some examples will indicate the state of our understanding how insects identify plants. For practical

reasons the sensory modalities are treated separately, although the animals appear to use inputs from **different sensory modalities** often simultaneously or in close succession. This is quite obvious when we observe a female Nymphaloid butterfly searching for her larval foodplant to lay eggs. She seems to inspect different plants visually for form and colour, sniffs and tastes with the antennae (and palps?), "drums" on the plants with front-leg taste sensilla (Dora Ilse, 1937) and, eventually, after a final surface probing with the ovipositing apparatus, deposits the egg.

Vision

During their mobile life stages nearly all insects use photoreception, be it with simple stemmata or highly developed compound eyes. A selective capacity for form and colour recognition can be deduced from behaviour studies. The **colour sensitivity** of the receptor cells reaches from the longer UV to the deep red, depending on the species. Many insect eyes, especially those of fast fliers, have an excellent **time resolution** which compensates for a less developed point resolution. **Form discrimination** is quite god in many insects, as was already shown in the classical studies of Mathilde Hertz, 1933, with honey-bees.

What concerns us here is, how the image, formed by the respective eye-types, is processed centrally? The caterpillar of the nun-moth **Lymantria monacha** (in its search for pine trees) prefers upright structures, thus it must be able to discriminate between horizontal and vertical structures and possibly in addition to be able to judge their width (Hundertmark, 1937). One may ask: can one stemma provide this information? Perhaps not, but a small set of stemmata would suffice. So far so good, but how and where is discrimination achieved and the decision made for up (=right) and flat (=wrong)? This extraction process is still unknown.

Recognition of complex and even subtle forms is greatly improved by the multi-facetted compound eyes and their highly developed ganglia in the adult insects. With respect to plant recognition a most impressive case is the egg-laying flight of a **Heliconius** butterfly (Gilbert, 1982). After finding the right species of passion-flower leaf, her decision to lay an egg or not depends among other criteria upon a visual inspection of the leaf. If she "sees" that there is already a yellow egg on the plant, she flies off, if not, she lays. Here the egg is the Gestalt stimulus guiding the avoidance behaviour. This habit seems to be the reason why some species of the **Passiflora** food-plants evolved the production of pseudo eggs, which suffice also to prevent egg laying by the butterfly - a most fascinating way of protection against herbivore attack.

Not only the form but also colour plays a role in plant identification: whiteflies (Homoptera: Aleurodina) avoid to settle in the presence of short wavelength illumination, 400 mu, but land on green light, 550 mu (Coombe, 1982). Moths of the African Armyworm (**Spodoptera exempta**) possess a four-colour visual system which reaches from the UV to the deep red and even functions in moonlight (Langer et al., 1979). One is tempted to interpret this capacity as a sensory basis for the moths when flying at night in a swarm to decide whether the vegetation on the ground is good for landing, mating and egg-laying.

Neuroethologists on the basis of the observed behaviour attempt to find with their repertoire of methods (ranging from structural to physiological and cybernetical techniques) the neural basis and the levels where such form, colour- and time-resolution of complex features is performed within the visual system. We can think of some kind of a "screen" on which the picture is projected by the receptor input. We do not doubt an **image formation**, but do not know the kind of transformation and abstraction taking place within the CNS, which is called feature-weighting. Furthermore, such extracted features which somehow are now manifest within the CNS, must be read out to respond to the "wanted Gestalt".

At present two functional concepts are discused: one is based on "**master cells**" on the top of the recognition pyramide, cells which by rather unknown mechanisms extract the Gestalt and then command the corresponding behaviour. The other concept deals with "**parallel networks**" and parallel computing of parts of the Gestalt, all feeding into the commanding circuit. So far, neither the master cell concept nor the network concept has a solid physiological basis (see for insect vision: Varju & Schnitzler, 1984).

Mechanoreception

The insect skin is an expanded mechanoreceptive "organ" which must also give rise to a central representation of some properties of the surrounding world. Mechanoreceptive sensilla (hairs, pegs, cones, cupulae, etc.) are found in the cuticle on most body parts. Those mechanoreceptors which are found on legs, antennae, palps, mouthparts and ovipositors are supposedly critical for the discrimination of plant surface properties (smooth, rough, hairy etc.). Such receptors have been studied electrophysiologically (Thurm, 1964) and the observed receptor properties would suffice to identify plant surfaces. To date, the central nervous representation and processing of the mechanoreceptor messages is not well known except that certain parts of the body are represented in certain central areas. Interestingly, many taste receptor hairs contain also a mechanoreceptor cell which indicates a cooperation of the two senses.

Olfaction

The insect sensors for volatile chemicals, the odorants, are found in a variety of types and numbers on the antennae, the palps and possibly also on other body parts. We ask: what is the specific odour image of a plant and how is it detected by the olfactory sense cells? Odour signals elicit the upwind searching behaviour and eventually add to the final process of identification of the plant. Some insects have been studied with respect to their attraction and orientation to plants (see Visser, 1986). The behaviour clearly shows that in the majority of cases several odorous components act synergistically, which means that the **chemical fingerprint of a plant is a complex of** compounds in even different concentrations.

If we now inquire again about the odour search image or Gestalt, we must (as in all sensory modalities) begin with the function of the receptor cells and their specificities. These cells are clearly the input units which send their impulse message to the brain, independent of whether their

membrane-bound receptor molecules have only a single or a variety of specificities.

Electrophysiological recordings have indeed in some cases shown that highly specialized receptor cells exist which preferentially respond to one plant compound, for instance in bark beetles (Mustaparta et al., 1974). However, in most cases we find a more complex situation, because single sensory cells which respond to plant odour are neither highly specialized (they respond similarly to a number of substances) nor particularly sensitive. Thus, it is difficult to classify these cells in type categories (in contrast to pheromone receptor cells) because the response "spectra" of such cells overlap to some extent when tested with a set of stimuli. It is important to stress this point again: the odour image of a plant is encoded by more than one receptor cell, namely in a set of receptor cells (called by Pfaffmann, 1941, the **Across-Fibre-Spectrum**). From it the CNS must read out the nature of the stimulus, that is the **odour image.**

But how is this image formed? What do we know of the fate of all the impulse messages which each of the individual receptor cells of the antenna sends via its axon into the **olfactory brain**? The first surprise came from neuro-anatomical studies by which a reduction of the number of neural elements - a functional convergence - of the rate of 100:1 or even more was found. The olfactory brain receives many more antennal nerve fibres than its intrinsic neurons send out to the protocerebral level. In the cockroach, perhaps the best studied example so far, nearly 200 000 antennal food-odour receptor cells send their axons (nerve fibres), and thus their individual messages to the olfactory brain. From here only 260 output fibres were counted which conduct the "food-message" to higher centers in the protocerebrum (Boeckh et al., 1984). Each of these 260 output neurons receives its respective information from one glomerulus, a characteristic spherical arrangement of nerve endings in the olfactory brain. The individual receptor cell fibres terminate here. Their message reaches more than one glomerulus either by direct projections or via local interneurones. The fibres of the output cells path to the mushroom bodies and terminate there with many endings.

Some of the output neurones were found to be influenced by mechanical stimuli (and light?), beside odour. Such influences are even more expectable at the next level, the protocerebrum and there, most of the neurones process **multimodal information.** It is assumed that from here the information is conducted in descending nerve fibres down the cord to segmental ganglia which are responsible for the control and generation of walking, flight, and other odour related motor activities.

But the central question in this context remains open: are there master cells among the output neurones of the olfactory brain or in the protocerebrum, cells which respond specifically to plant odours? And if so, how are these parameters encoded? At present, we cannot give a definite answer. Quite a few results point to network properties with parallel processing, and further studies at the level of descending neurones might provide an answer.

With respect to **odour guided orientation** we need to distinguish between 1) what an insect (e.g. a cockroach) does with its antennae, the

feelers, close to the odour source and 2) an "orientation" towards a distant odour source. This latter behaviour is a positive anemotaxis during which the odour signal only acts as a "go-command" while mechano-sensitive (for walking insects) or visual (for flying insects) sensors steer the approach (see Preiss & Kramer, 1986).

Short- and long term learning processes are particularly interesting and important phenomena in insect-plant relations. The **long-term learning** seems to operate in the chemo-receptor cells through changes in receptor properties and has been described as an induction process. The **short-term learning** is what we normally mean with learning such as in the honey-bee which even remembers a single (rewarded) odour exposure (Vareschi, 1971). Probably, very many insects learn their food plants quite well as it was now impressively shown for a butterfly learning specific flowers (Lewis, 1986). This learning capacity is, of course, particularly needed by all nectar feeders with changing nectar offers. But learning of the host odour might also be useful for a herbivorous insect if the plant odour changes due to a previous attack. Although a number of cases became known for such shifts in the composition of the plant compounds, little precise information is available on the corresponding behavioural or receptorial reactions.

Taste

The gustatory sensor is another important sensory modality for plant feeding insects, because taste probing is the final step in the identification of the diet. Taste-hair receptor sensilla with a tip pore occur on many parts of the insect body but the critical localities are the antennae, legs and mouthparts. These hair sensilla are accessible for electrophysiological recordings and have been extensively studied. With respect to our question for the recognition of the taste Gestalt of a plant, however, our expectations to find cells tuned to key stimuli have not been fulfilled. While specifically responding taste receptor cells for sugars and salts have been found everywhere, only a few receptor cell types were found which responded selectively to specific plant chemicals. If it is true that the pattern of secondary compounds in the tissues of a plant is the chemical gestalt which the taste receptors of the insects use for recognition, we have not found more than indications for a receptorial correspondence. Rather it looks as if such taste receptor cells which are specialized for a few or even only one plant compound allow the final decision following the broad information from the set of odour receptors. But the taste probing is - without doubt - essential for plant acceptance or refusal.

The central nervous representation of the taste message is as varied as the input pathways. Antennal taste fibres are - for example - contacting secondary cells in the dorsal deutocerebrum. Mouthpart taste-cells send their neurites to the suboesophageal ganglion and leg sensilla to the respective ganglia. Little is known as to what happens to such messages although striking reactions are known such as the proboscis response of the fly due to the sugar stimulation of just one single taste sensillum (Dethier, 1968).

Other modalities?

In addition to the sensory modalities so far discussed, some "strange" ones might also be important for the recognition of a plant. Since Lacher's (1964) work we know of extremely specific odour receptors for CO_2 on the honey-bee antenna. These organs were generally understood as sensors to control the atmosphere of the hive, but could also have a meaning for testing blossoms. We have recently learned that the labial palps of the moth **Rhodogastria** have a rich supply of CO_2 receptors which - interestingly - send their neurites to one of the basal glomeruli in the deutocerebrum (Bogner et al., 1986).

Finally, the long known triades of receptor cells in the locust pit-sensilla, which, respectively, respond to 1) dry air, 2) moist air, and 3) to changes of temperature, need to be considered (Waldow, 1970). Recently, it was discovered that at least some of the sensilla styloconica on the antennae of lepidoptera do respond as those of the locust (Steinbrecht & Kittmann, unpubl.). All these organs could likely supply further information to the "plant search command centre" in the insect brain, thus adding to the general neuroethological/ecophysiological challenge.

Let us now ask: what are the chances for a better of even total understanding of a plant recognition process in any insect? Not too great if we are realistic. Sensory processes of higher order are extremely complex and even the newest techniques would, at present, only help us to a partial insight. In this context I remember what Vincent Dethier told me some years ago (see also Hanson, 1983). He thought that the chemosensory and mechanosensory sensilla which control the feeding process of a caterpillar would give us a good chance for such a neuroethological approach. There is a total of less than 100 receptor cells (on the antennae, the palps and in the epipharynx) which are all localized and somewhat understood in their functions. With methods now available for neuron identification one needs to look for the central nervous terminals and connections to identify the image forming, processing and commanding units. Possibly, this system is sufficiently simple to be untangled by brave and enterprising students.

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DOES LEARNING PLAY A ROLE IN HOST LOCATION AND SELECTION BY GRASSHOPPERS?

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Grasshoppers have a highly developed locomotory ability, enabling them to move from plant to plant as they forage; in mixed stands they must encounter a wide range of sensory stimuli associated with plants. This, coupled with their large numbers of receptors and relatively advanced sensory capability (Blaney, 1975; Chapman, 1982) means that the sum of sensory information perceived by an individual is very complex over time.

Recent studies by Lewis (1981) and Chandra and Williams (1983) have demonstrated that the foraging behavior of the polyphagous grasshoppers studied may occur in a non-random pattern with respect to plant species composition. One possible mechanism to explain non-random foraging patterns is associative learning of visual, olfactory and/or gustatory cues associated with particular plants. This is perhaps most expected in highly mobile insects such as grasshoppers, which encounter a wider diversity of plant species during foraging than do less mobile insects. Since plants available to and encountered by mobile insects will also be variable in nutritional quality and in some cases contain toxic compounds, it would be of great value to individuals to learn to use cues that improve the ability to locate and select most suitable foods. It may be that the animal is able to associate the stimuli presented by a plant with some other aspects of plant quality, detected by reafferent input, which gives the animal information about its internal nutritional status. It has already been shown that rapidity of the rejection process with an unsuitable food plant can be improved by learning to associate information obtained by palpation alone with the stronger deterrent cues of plant sap obtained at biting (Blaney & Simmonds, 1985).

How, then, might the possible role of learning in host selection be further examined? Although field tracking studies can be of great value in assessing the patterns of foraging in insects, they cannot really serve to determine which physiological or behavioral mechanisms underlie them. The observed stochastic nature of the host selection behavior would be due in part to spatial and temporal differences in plant species composition, individual and within-plant variability, and a variety of physiological variables in the insects; ascribing behavior patterns to particular physiological mechanisms, including learning, is often impossible even when they are of signal importance. What is needed, then, are some relatively simple but relevant experimental designs that can demonstrate which processes are of importance in host selection. With this in mind we have embarked on a series of experiments to determine the potential importance

of learning.

In the first experiments (Bernays & Wrubel, 1986) the ability of the grasshopper **Melanoplus sanguinipes** to associate visual cues with the presence of food was tested. This was done by placing insects in an arena with two colored boxes, green and yellow. During a pre-training period in which the time spent in each box was monitored, a preference for yellow was found. Insects were then trained by placing wheat in the green box and allowing them to feed **ad libidum** for approximately 20 hours. After training, food was removed, boxes were replaced with clean ones and the insects tested for the number of visits to and the time spent in each box in the absence of food. During the first hour of post-training the proportion of total "box time" spent in green increased significantly as compared with pre-training. There was also a significant increase for the average visit length in the green box. The effect was short-lived; after an hour in the absence of food the original color preference for yellow was regained. The results indicated that **M. sanguinipes** individuals are able to learn to associate a visual cue with the presence of food.

The second set of experiments was carried out to determine whether grasshoppers were capable of learning to associate chemical cues with food. To assay for chemical learning, we took advantage of the fact that the desert locust, **Schistocerca gregaria**, has been shown to move upwind in a true anemotaxis in response to wind-borne grass odor (Kennedy & Moorhouse, 1969).

Newly molted fifth instar nymphs of related species **Schistocerca americana** nymphs were split into two groups and fed seedling wheat or fresch mint, plants with very different chemical profiles, for five days. On the sixth day they were deprived for four hours and tested blind in a wind tunnel (modified from Haskell et al., 1962) for their response to each plant odor. Tests were done in pairs, i.e., one individual from each food. For each pair a (+) score was given to the individual with the stronger response. Similar experiments were conducted with insects that had been fed wheat or mint for one day, or for one hour.

In the five-day feeding experiment mint-fed insects had a significantly larger proportion of (+) scores in response to mint odor (0.72) than did wheat-fed insects (0.28), ($n=73$, $X^2=13.16$, $P<0.0005$). The same pattern held for a one-day experiment: proportion of mint-fed with (+) score = 1.00 ($n=8$, $X^2=8.00$, $P<0.005$). A one-hour feeding experiment showed no significant difference between the two groups.

These results suggest associative learning of a plant olfactory cue; further work is required to control more precisely for the degree of food deprivation in each group. Work is also in progress to test the insect's ability to learn and respond to mint secondary compounds alone, since these non-nutritive chemicals couls provide the cues utilized in an associative learning process.

Learning is being reported in various herbivorous insects (e.g., Dethier, 1980; Lewis, 1986; Papaj & Prokopy, 1986; Traynier, 1986). If extensive learning abilities are found to be widespread in herbivores, are they greater in more mobile species, more polyphagous species, or more neurally complex species such as acridids?

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PLANT ODOUR PERCEPTION IN THE COLORADO POTATO BEETLE: CHEMOATTRACTION TOWARDS HOST PLANTS

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Host selection behaviour of phytophagous insects is divided in (1) searching for host plants, and (2) host plant recognition. In these two phases insects are exposed to very different stimulus conditions. In host plant recognition, the insect's conclusion to accept or reject a plant for feeding or oviposition, is reached upon the perception of a number of stimuli being associated with the one plant under inspection. When searching for host plants, insects walk of fly around, and, at a first glance, their movements seem to be at random. There is an increasing evidence, however, that insects direct their movements towards host plant patches as they perceive olfactory and visual plant characteristics from a distance (Visser, 1986). In this respect the term attraction is often used.

Chemoattraction

We have previously reported on the chemoattraction of the Colorado potato beetle, **Leptinotarsa decemlineata** Say, to its host plant potato, **Solanum tuberosum** L.. Wind-borne potato plant odour elicits a positive anemotactic response in the beetle (Visser, 1976). The perception of both odour and wind is indispensable for the release of the odour-conditioned anemotaxis.

The next step was to isolate the host plant odour by steam distillation and direct vapour sampling techniques. GC-MS analyses of these samples identified, among other components, cis-3-hexen-1-ol, cis-3-hexenyl acetate, trans-2-hexenal, trans-2-hexen-1-ol, and 1-hexanol (Visser et al., 1979; Visser, 1983).

In addition, we conducted studies on the olfactory receptors. The identified C6 components, their isomers, and members of other classes of plant volatile compounds were screened for EAG activity (Visser, 1979). The data led us to conclude that the antennal receptor system of the Colorado potato beetle is sensitively tuned to the reception of the C6 components which are present in potato leaf odour. Furthermore, we recorded receptor activities of single olfactory sensilla when stimulated by individual C6 components (Ma & Visser, 1978; Visser, 1983). The receptor cells respond differentially to the class of C6 components, and, in this way, they may discriminate between individual components.

Additional support for the idea that the C6 components are involved in the chemoattraction of Colorado potato beetles, was obtained through wind tunnel studies (Visser & Ave, 1978). None of the individual C6 components elicit odour-conditioned positive anemotaxes in the beetles. When

individual components are artificially mixed with the odour of potato plants, however, its prevents the beetle's upwind orientation. The induced change in concentration ratios between components in the leaf odour blend eliminates the attractiveness of the host plant odour. Thus, chemoattraction of the Colorado potato beetle depends on the ratios between C6 components.

The C6 components which were identified in potato leaf odour, are present in all green leaves, and their concentrations differ between plant species (Visser et al., 1979). A relatively large number of phytophagous insects have now been tested on their antennal sensitivities for these components (Visser, 1983, 1986). All leaf feeding insects respond to the C6 components, and one might call this class of chemicals the green odour. The specificity of the green odour (the tint of green) is set by the concentration ratios between constituents.

Biological factors affecting the range of chemoattraction

Our recent research focusses on the searching efficiency of the Colorado potato beetle. In a more precise sense, we are interested in the biological factors affecting the range of the insect's olfactory orientation. We restrict ourselves to biological factors, and will not discuss the impact of physical factors on the range of chemoattraction, such as odour release and dispersion (Visser, 1986). In brief, biological factors include: (1) the intensity of the odour-conditioned anemotaxis, (2) the integrity of the chemical message, and (3) the insect's responsiveness to host plant odour. In order to recognize the significance of these factors we use the Colorado potato beetle as a model to study the chain of stimuli - receptors - central nervous system - behavioural patterns.

1. Intensity of the odour-conditioned anemotaxis

We realized that further behavioural analyses ought to contain precise information on (a) the intensity of the odour-conditioned anemotaxis, and (b) the steering mechanisms involved in orientation. For these reasons a locomotion-compensator was constructed in our department, with the help of the original designers E. Kramer and P. Heinecke (Max-Planck Institut fuer Verhaltensphysiologie, Seewiesen, FRG). A full description of the equipment will be presented elsewhere (Visser & Thiery, in prep; see Thiery & Visser, 1986a). For the present discussion it is sufficient to report that this instrument automatically compensates all movements of an insect freely walking on a large sphere. At the same time pulses are generated, and analysed by a microprocessor in order to quantify the beetle's orientation. The locomotion-compensator is positioned at the outlet of the contraction of the wind tunnel described by Visser (1976).

Experiments were carried out with starved females as to assess their behaviour under (a) control conditions, (b) at wind stimulation (80 cm/s), and (c) stimulation by wind-borne host plant odour. Under all conditions the light intensity on top of the sphere was set at 1750 Lux by means of two high-frequency illumination units which were suspended at the ceiling of the observation room.

In every second angle and speed were calculated; representative

distributions are shown in Figures 1 and 2.

Under control conditions angles are evenly distributed, at wind stimulation downwind classes dominate, and in the combination with host plant odour the angle of movement is restricted to upwind directions (Fig. 1). Wind stimulates the beetle to walk faster (Fig. 2). When a beetle is simultaneously stimulated by wind and host plant odour the variation in mean walking speed, that is the standard deviation, is reduced (Fig. 2). This second effect of odour stimulation is related to the straightness of the track since minor angle deviations will cause only small reductions in walking speeds (Visser & Thiery, in prep).

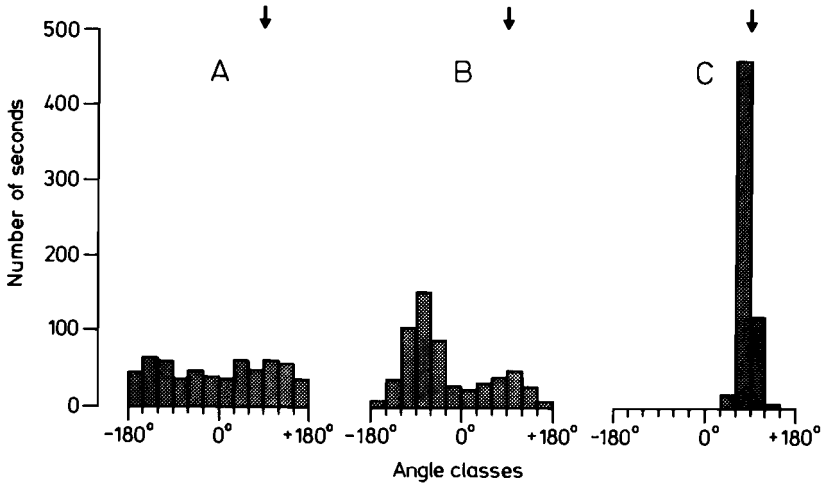


Figure 1. Distribution of angle classes of three 10-min. walking tracks of one Colorado potato beetle. A: control conditions; B: stimulated by wind; C: stimulated by wind carrying potato plant odour. Arrows: position of wind tunnel.

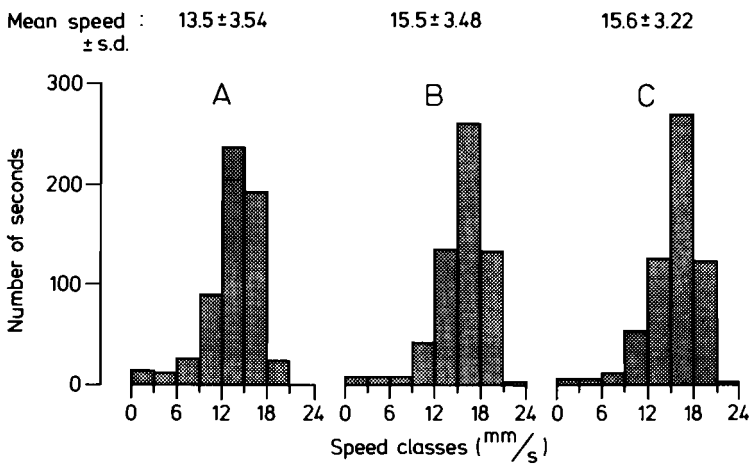


Figure 2. Distribution of speed classes. See legend of Fig. 1.

A large number of walking tracks were analysed by defining descriptive measures like: vector length, vector angle, upwind length, total straightness, walking speed, mean straightness, circling time, angle preference, and upwind time (Visser & Thiery, 1985, in prep.; Thiery & Visser, 1986a, 1986b).

The first striking feature of tracks is the amount of circling. In control conditions beetles walk in circles for long periods. The time spent on circling is reduced by wind stimulation, and circling behaviour is nearly absent in wind carrying host plant odour. The second characteristic of tracks is the angle preference which is defined as the angle preference during straight walks (corrected for circling). In control conditions the angle preference is small. Wind releases anemotactic orientation at angles that are, about every 2 minutes, changed in a new direction. Wind in combination with host plant odour elicits a constant upwind angle preference.

Under all conditions, that is included of controls, descriptive measures fall into two groups denoted by the motor patterns: (1) walking circular or (2) keeping direction. Variations in orientation between individuals, and the variations within one individual during prolonged testing, are explained by the varying proportions of the motor patterns walking circular and keeping direction. Two types of steering mechanisms control these motor patterns. Idiothetic control or walking circular is considered as an internal programme. Allothetic control of keeping direction is a programme which reduces the asymmetrical input of an external stimulus. Thus, the intensity of the odour-conditioned anemotaxis is regulated by the combined actions of idiothetic and allothetic control (Visser & Thiery, 1985, in prep.)

2. Integrity of the chemical message

In diverse vegetations or mixed croppings wind turbulence will blend volatiles from host and nonhost plants. The integrity of the chemical message, therefore, may by changed or even lost. We tested this hypothesis by combining potato plants with wild tomatoes, **Lycopersicon hirsutum** f. **glabratum** C.H. Mull, or cabbage, **Brassica oleracea** L. var. **gemmifera** D.C., in the upwind compartment of the wind tunnel (Thiery & Visser, 1986a, 1986b). In these odour blends Colorado potato beetle do not show odour-conditioned anemotaxes, their responses are identical with those in pure wind. The attractiveness of host plant odour is neutralized by blending with nonhost plant odours.

It is noteworthy that these behavioural observations confirm our conclusions on the perception of plant odour blends. Intracellular recordings of deutocerebral neurones revealed two reaction groups for (a) the detection of C6 components, and (b) the detection of incorrect ratios (De Jong & Visser, this volume).

3. Insect's responsiveness

The insect's responsiveness is changed as a result of feeding experience (Visser & Thiery, 1986). We compared two groups of beetles. One group of newly-emerged females was starved, while another group of females

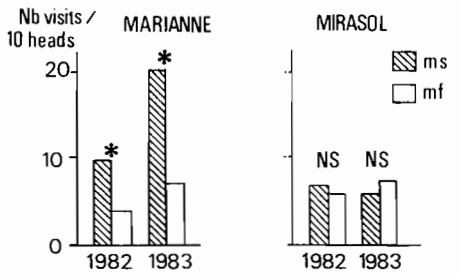
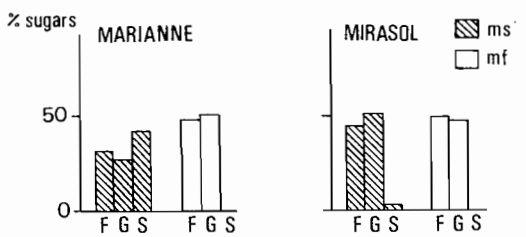
MARIANNE	MIRASOL	
5-6 qx/Ha	10-13 qx/Ha	YIELDS certified seeds
328	1327	seeds/head (♂ sterile line)
		BEHAVIOUR forced pollination under tunnels
qualitative and quantitative genotypic differences : 8 compounds among 130 (10% of the whole aromatic blend) 20 compounds among 250 Bee takes into account a limited fraction of the total extract = limited aromatic pattern to make its choice.		AROMA Chemical analysis Combined chemical and behavioural analysis
 Glucidic pattern corresponding to each genotype (Fructose, α and ß Glucose, Sucrose) Sucrose is a decisive factor for selective behaviour		NECTARS (GLUCIDS) Chemical analysis

Figure 1

was fed for 2 h on potato leaves, and then starved. Experienced beetles show a significant increase of upwind responses compared with non-experienced beetles.

We illustrated in the present paper that the range of the insect's chemoattraction is not solely affected by physical factors. The elucidation of biological factors implicates detailed analyses of the insect's behaviour in the laboratory. Further field experiments are needed as to appreciate the chemoattraction of insects, and to proceed with the development of intelligent methods of insect pest control.

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CHEMICAL BASIS OF PLANT-INSECT RELATIONSHIPS: THE "HONEY-BEE - SUNFLOWER MODEL"

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1. Introduction

Among pollinating insects, honey-bees have a particular economical interest for crop improvement through hybrid seed production. In order to analyze plant-honey-bee relationships leading to entomophilous pollination, basic studies of foraging behaviour and of specific stimuli releasing this behaviour must be set up. Such studies involve multidisciplinary approaches (behavioural and chemical analyses) carried out at differnt levels (field and laboratory experiments).

The present work deals with honey-bee - sunflower model. The sunflower has recently become world's second largest oil-seed crop, since the discovery of a male cytoplasmic sterility (Leclercq, 1969) has allowed creation of controlled hybrids. In sunflowers, hybrid seed production strictly depends on entomophilous pollen carrying from male to female lines. But field observations point out that a selective foraging behaviour among some sunflower lines may occur (Freund & Furgala, 1982).

Such behaviour is linked to a learning phenomenon where plant chemicals are mainly involved (Masson, 1982): while foraging, workers associate food uptake (nectar, pollen) with plant aromas, which are then memorized as orientation cues.

Therefore, we try to determined how far honey-bees are likely to discriminate among sunflower genotypes, using scents associated to food reinforcement; this should lead to a definition of molecular criteria of plant attractiveness, likely to become tools for plant improvement through entomophilous pollination.

2. Plant material

Experiments are carried out with two pairs of parental lines producing commercial hybrids Marianne and Mirasol. Seed yields of these hybrids are widely different, respectively 5-6 qx/ha and 10-13 qx/ha (AMSOL data, 1984).

3. Behavioural studies under forced pollination conditions

Experiments take place under pollen-proof tunnels; each tunnel covers one pair of parental lines, following rows in 1982, and following a check-patterned disposal in 1983, in order to show a possible "row effect" on forager choices. For each pair of parental lines, pollination is carried out by a honey-bee colony of about 10 000 workers. Forager distribution is recorded during flowering for a sample of 40 male heads and 40 female

heads.

It appears that, whatever disposal is used, foragers exhibit a selective behaviour among Marianne parental lines, with a significant preference for the female line, whereas they are randomly distributed on Mirasol parental lines. Such behaviour points out forager ability to discriminate among different genotypes and is directly related to the differences of seed yield previously mentioned.

4. Role and nature of allelochemicals implied

4.1. Olfactory cues

Only a few works have been carried out on sunflower chemistry, in contrast to other vegetal groups of agronomical interest.

Our work focuses on chemical analysis of sunflower volatiles combined with behavioural studies, to define compounds responsible for sunflower attractiveness towards honey-bees.

4.1.1. Aroma collection and analysis. Techniques of aroma trapping and extraction were set up on sunflower by Etievant et al. (1984). Two techniques were followed in parallel:

- a technique of headspace trapping applied to living sunflower heads, leading to a qualitative extraction, very close to natural plant emissions.
- a technique of solvent extraction by washing plant material with dichloromethane, leading to isolation of great amounts of a very aromatic oil.

Volatile constituents are then separated from the non volatile fraction by a molecular distillation under high vacuum conditions (Azar, 1982).

After extractions, chemicals are separated by gas chromatography and identified by coupling gas chromatography and mass spectrometry. Results obtained from these analyses are detailed by Etievant et al. (1984).

4.1.2. Results. As is usual in most plant aromas, sunflower aroma appears as a complex blend of volatiles. The comparison of compounds identified after both techniques of extraction points out a very similar composition of the aromatic blend, which shows the validity of both techniques (Etievant et al., 1984). But dichloromethane extraction provides a quantitative extraction of volatiles and a much more concentrated extract (1 per 6, related to behavioural responses obtained with each extract); that is why solvent extracts were kept for the following behavioural study (cf. 3).

Among hundreds of compounds detected by gas chromatography, 84 are particularly representative and have been indexed, and 58 are identified (Etievant et al., 1985). Moreover qualitative and quantitative differences appear in aroma composition of different sunflower genotypes.

These date are confirmed by the compared analysis of chromatographic profiles of aromas collected by head-space trapping form heads of Marianne and Mirasol parental lines (Pham-Delegué et al., in prep): significant inter-genotypic differences appear for 7 compounds among 130 for Marianne

parents, and for 20 compounds among 250 for Mirasol parents. Thus, concerning Marianne parental lines, honey-bees are able to discriminate among genotypes on the basis of a few compounds among a complex chemical blend. Such results are related to those obtained by combining chemical and behavioural analyses under controlled conditions (cf. 3).

4.2. Gustatory cues

Honey-bee foragers usually visit sunflower heads for nectar collection.

Numerous works were carried out on the role of nectar production in sunflower attractiveness towards honey-bees. But quality of sugars and their relative proportions were not taken into account, so we set up experiments to analyze qualitative aspects of nectars.

4.2.1 Nectar collection and analysis. Nectar collection carried out with glass pipettes, from 20 florets per heads and 10 heads for each parental line of Marianne and Mirasol, throughout flowering.

Amounts of nectar collected are directly read on the pipettes; samples are then kept in a freezer (-25°C) for later analyses.

Nectar amounts are sometimes very slight, on these occasions a precise and sensitive analysis is needed. We carried out a gas chromatography previously used by Bosi (1973) which allows the estimation of relative proportions of each sugar from partially dehydrated and derivatized samples of nectars (Fonta et al., 1985). Currently, this technique is modified following Black and Bagley (1978) who introduce an internal standard (triphenyl ethylene) to determined the real proportions of each sugar of the nectars (Pham-Delegué et al., in prep).

4.2.2. Results. It appears that, for the genotypes considered, total amounts of nectar or total amounts of sugars in the nectars cannot be related to forager preferences (Fonta et al., 1985). But, considering the glucidic composition of nectars, a "glucidic pattern" characterized each genotype: fructose, glucose and sucrose were identified with proportions proper to each genotype, over several years and at different places (Pham-Delegué et al., in prep.). Moreover, it appears that sucrose is mainly involved in bee choice: thus, preference for the female line of Marianne relates to high amounts of sucrose, while the male line, equally supplied in fructose and glucose, has nearly no sucrose. When both parental lines have a lack of sucrose (Mirasol case) honey-bees do not significantly disciminate among the two lines.

5. Combined chemical and behavioural studies

In order to define, in a more analytical way, the roles of the different chemicals involved in foraging behaviour, the foraging situation is simulated in controlled conditions, with a bioassay using an artificial flower feeder device (Pham-Delegue, 1983). The bioassay is based on an associative conditioning between a sugary reinforcement (= unconditioned stimulus) mimicking the nectar and an olfactory signal (conditioned stimulus) constituted by the floral aroma; this conditioning is applied to

a whole colony flying freely in a flight room of 12 m^3.

After a conditioning period where foragers memorize the scent associated with the food source, a choice is given between the conditioning scent and a control or another scent, without any reward. Then forager distribution allows an appreciation of their ability to recognize the conditioning scent or to discriminate among the different scents.

Using this bioassay combined with chemical analysis of sunflower aromas, we analyzed how a complex chemical information may be used by foragers for selective orientation towards a food source (Pham-Delegue et al., 1986).

Aromatic blends from dichloromethane extraction are chemically fractioned into polar and apolar fractions; these are successively presented to bees, and compared to the global extract as a conditioning stimulus. Behavioural responses show that the apolar fraction is significantly discriminated from the conditioning global aroma, while the polar fraction is confused with the global aroma: thus the polar fraction is considered by honey-bess as very close to the global aroma. Then three fractions of the polar fraction, obtained by preparative chromatography, are successively presented to bees, compared to the polar fraction used as the conditioning stimulus. It appears that only one fraction, composed of about 20 compounds, nearly all identified (Pham-Delegue et al., 1986), allows the recognition of sunflower aroma. This limited fraction can be considered as an active fraction of sunflower aroma.

So it appears that honey-bees have a selective behaviour based on a "limited aromatic pattern" of plant aroma.

In the previous experiment, the reward is kept constant while olfactory signals vary, this allows an analysis of their role. Alternatively, the bioassay can be adapted to the study of gustatory parameters involved in bee choice. After a gustatory conditioning to a given sugary solution, it is possible to analyze bee preferences among different pure sugars or sugary blends, among different concentrations of sugary solutions.

Previous experiments were carried out with honey-bee populations, in semi-natural conditions. In order to focus on individual behaviour of foragers and to analyze more precisely quantitative aspects of the active volatiles, we set up a bioassay based on an orientation behaviour in a multiple olfactory choice situation. This assay uses a four armed airflow olfactometer adapted from the device set up by Vet et al. (1983) for **Trichogramma** behaviour observation. This device allows a better control of olfactory stimulation, applied to strictly controlled individuals of known genetic origin, physiological state, age and olfactory experience.

In previously described bioassays there was either a delay between the chemical analysis of olfactory and gustatory signals or only one or the other were assayed. A recent development of our work is to carry out a simultaneous coupling of chemical analysis and electrophysiological or behavioural tests (Thiery et al., in prep.).

6. Applied developments

Concerning aromas. Forager distribution on different sunflower genotypes

under tunnels, combined to chromatographic profile comparison of corresponding genotypes reveals the honey-bees' ability to make a choice based on a limited range of chemicals. These data are confirmed when combining chemical analyses with a bioassay under controlled conditions, which shows that among hundreds of compounds of plant aroma, honey-bees may use a limited fraction of the whole blend, to identify the total aroma.

It appears that the significant part of the complex olfactory information is limited to a few compounds (the number of which could be reduced by simultaneous coupling of chemical and biological techniques), most of them being identified. The identification of attractive blends and of aromatic patterns specific to given genotypes may be of great interest for plant breeders. Thus it is possible, by combining genetic and chemical analyses, to elucidate the genetic basis of plant volatile biosynthesis. So, by this method it should be possible to create varieties producing attractive aromatic blend, or on the other hand to develop plant chemical resistance towards pests, selecting some terpenoids, as suggested by Gershenzon et al. (1981) in **Helianthus** genus. Such a genetical control may also allow to limit olfactory cues of discrimination among genotypes, leading to cross pollination improvement.

Concerning nectar. Compared to volatile blends, glucidic composition of nectars is much less complex. In comparison to many floral nectars, sunflower nectar is mainly constituted of sugars, with rather simple qualitative profiles. Such glucidic profiles are genetically controlled, and positive correlations appear between honey-bee visits to a genotype and amounts of sucrose. It may be possible then, to control glucidic composition of nectars through plant selection to increase genotype attractiveness and/or to make a screening of genotypes before coupling them for hybrid seed production, leading to a more randomized distribution of foragers. However, other nectar constituents, such as amino acids and ions, may be of importance in pollinators' food choice; these criteria are currently taken into account.

To summarize (Fig. 1): such multidisciplinary studies carried out at different levels, lead to a better knowledge of allelochemicals involved in plant-pollinator relationships. It may then be possible to integrate certain plant molecular criteria responsible for bee attraction and choice, into plant breeding programs.

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INCONSTANCIES OF CHEMORECEPTOR SENSITIVITIES

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1. Introduction

The act of host selection is a critical element in all insect-plant relationships and has therefore drawn much attention from entomologists interested in insect behaviour and its physiological basis. From these studies it has been inferred that in most cases chemicals play a major role in host plant recognition (V.G. Dethier, this symposium). To extend our understanding of this recognition process we need three types of information. First, we need information on the identity, concentration and location of the key chemicals in the plant. Second, we have to know the sensory coding mechanism, which the insect uses to transform the (often complex) chemical stimulus into a neural message which is sent to the brain, and, third, we need to understand the central processes, underlying the translation of sensory input into feeding behaviour.

The role of taste in host plant recognition is particularly amenable to this type of study, and lepidopterous larvae appear to be ideal insects, since the major gustatory input occurs through only eleven pairs of taste receptor neurons. Two sensilla styloconica on each maxilla each contain four neurons and the epipharyngeal organ is innervated by three pairs of chemoreceptive neurons. The styloconic receptors of a number of caterpillar species have been characterized in terms of their "best stimulus". Thus cells stimulated by, for instance, sugars, inositol, amino acids or deterrents have been established (Schoonhoven, 1986). In several cases also the relationship between stimulus concentration and receptor activity has been determined with a fair degree of accuracy (e.g. Simmonds & Blaney, 1984). Once the qualitative and quantitative responses of the receptors are known, sensory input can be correlated with behavioural output as manifested by either food preferences (in choice experiments) or feeding intensity (in no-choice situations). Such correlations may reveal some of the principles governing central nervous integration processes. Using such methods it was found that, under certain conditions, feeding intensity is indeed proportional to neural activity in specific receptors (e.g., Ma, 1972; Blom, 1978). Additionally it can be demonstrated that inputs from different receptors are evaluated in the CNS in quantitatively different ways (Schoonhoven & Blom, unpubl.).

However, before the quantitative relationships between sensory input and behavioural output can be explored in more detail, it is important to know whether, under given conditions, receptor sensitivity remains

constant, or if not, what factors cause it to change. This paper concentrates on the problem of receptor variability. It is known that in vertebrates the CNS may modulate receptor functioning by way of efferent neurons. Although evidence for the presence of efferents innervating insect chemoreceptors is uncertain, this does not necessarily imply that their sensitivity is constant under different conditions. Indeed there is a growing body of literature indicating that insect chemoreceptors may show variations in their sensitivity (Blaney et al., 1986).

2. Experimental

To investigate which factors may affect receptor sensitivity larvae of the armyworm (**Spodoptera littoralis** Boisd.) were reared on different diets and the receptor responses to a number of chemicals were recorded. In addition two other experimental variables were investigated: the influence of short starvation periods and the effect of time of the day on receptor sensitivity. Total neural activity of all four receptors in each sensillum styloconicum was counted during one second.

All three experimental variables appear to affect receptor sensitivity. Figure 1 shows that larvae reared on cabbage fire significantly higher impulse frequencies in their medial sensillum when tested with sinigrin than larvae reared on an artificial diet. This indicates that receptor sensitivity is not a constant and static feature, but may vary, depending on past experience, of which diet composition is a significant aspect.

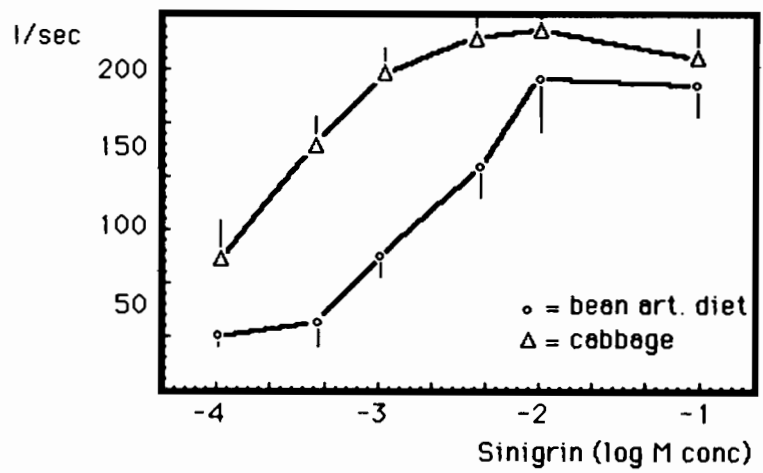

Figure 1. Total neural response (impulses/sec ± S.E.M.) of medial sensillum styloconicum of fifth instar larvae of **Spodoptera littoralis** when stimulated by sinigrin solutions of varying concentrations. Insects were grown on an artificial bean diet or on cabbage leaves.

Another variable influencing receptor responses is related to time of the day. When results obtained in the morning were compared to those collected in the late afternoon, that is with a time difference of about

seven hours, all other experimental conditions remaining constant, it appeared that sensillar responses in the morning were in several cases stronger than in the afternoon (Fig. 2). Apparently receptor sensitivity is modulated by some diurnal rhythm, either directly, or via the effect of that rhythm on feeding activity; cause and effect are not yet unequivocally established. Note that the data shown in this figure also feature striking differences in response intensities between diets.

As a third variable we have investigated the effects of increasing starvation periods on receptor sensitivity (Fig. 3). In this experiment insects were tested at various periods after removal from their food.

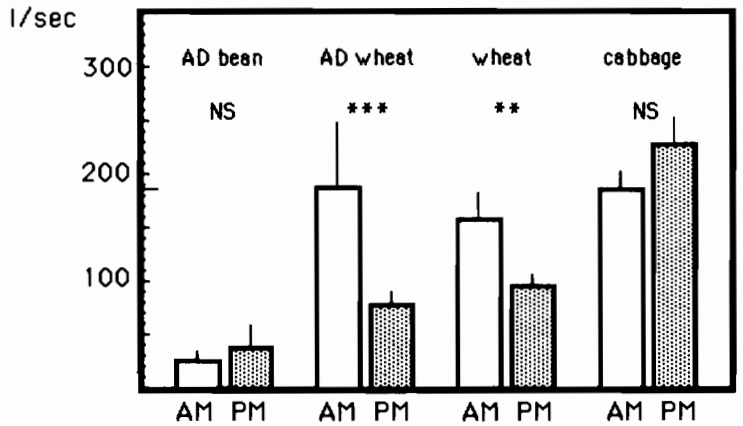

Figure 2. Responses of lateral sensillum styloconicum of fifth instar larvae of **S. littoralis** (day 2) to 1 mM sinigrin in 50 mM NaCl. Insects were reared in artificial diets or on wheat or cabbage leaves. Insects were tested in the morning or late afternoon. N=15

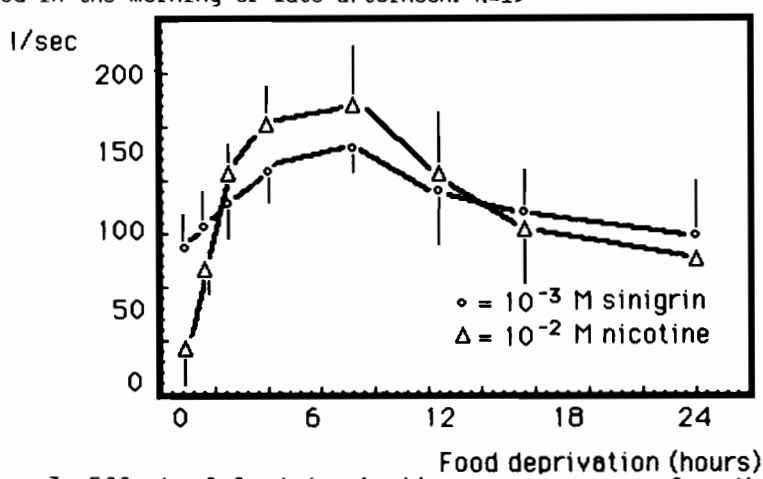

Figure 3. Effect of food deprivation on responses of medial sensilla styloconica of **S. littoralia** larvae, reared on aritificial wheat diet. N=15

It may be seen that sensillar response intensity increases with increasing starvation periods, and reaches a maximum after about eight hours of food deprivation, then declines. The change in sensitivity with time differs between compounds.

3. Conclusions

It can safely be concluded from the data presented that chemoreceptor sensitivity is not fixed and constant property. On the contrary it may vary with the type of food the insect has been reared on, the time of the day, and the state of hunger. The question that immediately arises is whether the altered sensory input does affect feeding behaviour or not. If a close relationship exists between receptor activity and food preferences or feeding intensity, then receptor changes would be reflected in modifications of feeding behaviour. The results of an earlier study (Schoonhoven, 1969), on tobacco hornworm larvae, indicate that this is the case. By feeding these insects on diets containing salicin, the sensitivity of their deterrent receptor was significantly reduced (Fig 4). The same phenomenon was observed when insects were reared on tomato leaves with their petioles in a salicin solution. When the latter insects were offered non-host plants, which normally are rejected by most caterpillars, e.g. cabbage or dandelion, these plants now evoked a higher feeding response than in control insects. Apparently the reduced sensitivity of the deterrent receptor corresponds with alterations in food acceptability.

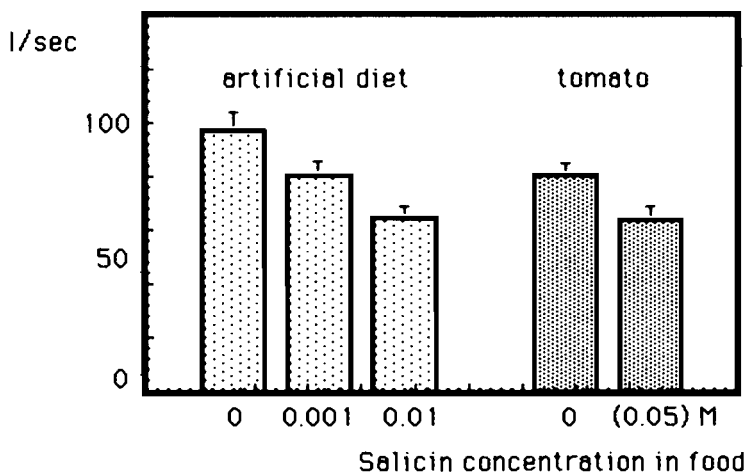

Figure 4. Responses of the deterrent receptor in the lateral sensillum styloconicum of fifth instar larvae of **Manduca sexta** when stimulated by 10 mM salicin. The insects were reared on artificial diets containing 0, 1 or 10 mM salicin (left) or on tomato leaves with their petioles immersed in water or in 50 mM salicin solutions (right) (date from Schoonhoven, 1969).

The foregoing results contain a caution, but also reveal a new perspective of sensory function. First it becomes obvious that when attempts are made to decipher the sensory code, it is essential to use very

rigorous experimental procedures and to standardize carefully as many factors as possible.

A second conclusion is that under natural conditions the CNS of an insect does not receive a constant and predictable message. On the contrary this message appears to be modulated by many variables, including previous experience, internal physiological state (e.g. state of hunger, time of the day, and possibly other hitherto unknown variables as well. This may complicate the interpretation of the sensory message by the brain considerably. Conceivably, however, the capacity to modulate receptor sensitivity should not be considered as a disturbance of central integration processes, but as a sensory characteristic which facilitates subtle responses to a large number of external and internal variables. When decision processes are not restricted to the CNS, but rather involve also other components of the neural system, i.e. receptors, a more efficient and diverse use can be made of the total neural assembly an insect possesses.

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ELECTROCHEMISTRY OF PHYTOCHEMICALS AS REPELLENTS OR ANTIFEEDANTS TO INSECTS

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1. Introduction

A primary objective in chemoreception studies is deciphering the information code by which sensory (afferent) input energy is transduced into motor (efferent) output energy (whole insect behavior). Such a code is simply the set of rules which governs the mapping of the input variable onto the output variable (O'Connell, 1981). Encoding mechanisms require only the rules are applied uniformly and that they lead to a distinctive outcome (e.g., avoidance behavior). Such an encoding mechanism, which can be readily expressed quantitatively in millivolts, seems to be emerging from our investigations of **Scolytus multistriatus** (smaller European elm bark beetle) and **Periplaneta americana** perceptions of 1,4-naphthoquinones as allelochemicals (Norris, 1986). Experimental elucidation of this apparent code is described here.

2. Procedure

Detailed descriptions of utilized methods and materials have been published (Rozental & Norris, 1973; Norris & Chu, 1974; Norris, 1985, 1986).

2.1. Behavioral assays

2.1.1. **Scolytus multistriatus**. The behavioral assay with this bark beetle adult was a two-choice test as described by Norris (1986). Each replicate assay involved 25 newly emerged adults per petri dish arena. The bioassay was run 48 h in darkness at 25°C. Amount of feeding per assay disk was measured in mm^2.

2.1.2. **Periplaneta americana**. The employed two-choice "avoidance" behavioral assay was with adult males, and utilized the inverted cardboard tub arenas as detailed by Rozental and Norris (1975). Ten adult males of biotype LAB or WARF were used per replicate 2-tub assay. Overhead light was employed to drive cockroaches into one, or the other, of two (i.e., 1,4-naphthoquinone- or chloroform solvent-treated) inverted tub arenas which had uniform "doors" cut every 90° on their larger diameter end. Assays were run for 3 h with cockroach counts-per-tub being taken each 30 min; the 10 cockroaches per 2-tub assay were randomly dispersed in the enclosing lighted assay chamber after each counting.

2.2. Electroantennogram (EAG) assays

The electroantennogram (EAG) assays of 1,4-naphthoquinone allelochemical (repellent or antifeedant) activity were run with antennae from adult male **P. americana** as detailed by Norris and Chu (1974). The standardized test determined how much inhibition (i.e., % in millivolts) occurred in an uniform amyl acetate-excited EAG when the antenna was pretreated with known moles of a specific allelochemic 1,4-naphthoquinone.

2.3. Polarographic electrochemical (E 1/2) assays

The characteristic millivolt E 1/2 shifts induced in vitro in standardized (i.e., 100 ug/cm^3) aliquots of nerve membrane-associated protein isolated from 100 pairs of antennae from biotype LAB or WARF adult male **P. americana** by the indicated moles of a 1,4-naphthoquinone were measured at 25°C using a Radiometer PO4 Polariter polarograph with a dropping mercury electrode (d.m.e) and a saturated calomel electrode (s.c.e.) as detailed by Rozental and Norris (1973) and Norris (1985). Such proteins were solubilized from nerve membrane fragments using two extractions with 0.6% Triton X-100 detergent in 0.9% NaCl. Protein concentration was determined according to Lowry et al. (1951).

Crystalline bovine serum albumin (BSA), with and without 0.6% Triton X-100, was used as a standard protein control for polarographic reactivity with 1,4-naphthoquinones or other chemicals. Saline-soluble proteins from the homogenized insect antennae also were used as controls.

3. Results

3.1. Behavioral analyses

Repellent and antifeedant activities of the several tested 1,4-naphthoquinones on adult **S. multistriatus** and **P. americana** indicated that the order of relative effectiveness was 5-OH > 1,4- > 2-OH > 2-CH_3.

The observed times (X) of moles of a given 1,4-naphthoquinone required for > 99% **P. americana** avoidance behavior, (X) M_{99}, where that quantity for 5-OH and LAB, $2x10^{-5}$ M, was set at one (1)X, is as follows, respectively, for LAB and WARF: 5-OH, 1 and 5X; 1,4-, 25 and 40X; and 2-CH_3, 500 and 1000X. These two observed values for each naphthoquinone are different ($P<0.01$).

The increasing repellency of increasing concentrations of 2-methyl-1,4-naphthoquinone (menadione) to each biotype (LAB or WARF) of **P. americana** was described by equations for three overlapping linear regressions (also see Norris, 1985).

3.2. EAG analyses

Mean maximum % inhibition (max % Inh EAG) of the standardized amyl acetate-excited EAG of both **P. americana** biotypes (LAB vs. WARF) was similar ($P<0.01$) for each 1,4-naphthoquinone. Observed mean maximum % inhibitions for LAB and WARF, respectively, were: 5-OH, 50 and 48%; 1,4-, 42 and 39%; 2-OH, 34 and 30%; and 2-CH_3, 25 and 23%.

3.3. Polarographic E 1/2 analyses

Mean maximum E 1/2 shifts induced in vitro in standardized aliquots of nerve-membrane-associated protein from LAB and WARF biotypes of **P. americana** were quantitatively similar ($P<0.01$) for each 1,4-naphthoquinone. Observed mean maximum E 1/2 shifts in millivolts for LAB vs. WARF, respectively, were: 5-OH, -30 and +33; 1,4-, -21 and +24; and 2-CH_3, -15 and +13. These induced E 1/2 shifts in LAB versus WARF did differ by the former being more-negative (-) and the latter more-positive (+) than the untreated control value, -1860 mV. The 1,4-naphthoquinones did not cause detected E 1/2 shifts in BSA, or saline-soluble (control) neuronal proteins.

4. Discussion

All parallel studies of 1,4-naphthoquinones as repellents or antifeedants to **S. multistriatus** and **P. americana** have supported similar molecular property-biological activity relationships. The consistently observed order of biological activity is 5-OH > 1,4- > 2-OH > 2-CH_3. As detailed by Norris (1986), these 1,4-naphthoquinones all react (bind) with a redox-complex "receptor" protein in the dendritic membrane of primary chemosensory neurons in sensilla; and all but 2-CH_3 (menadione) further interact with (i.e., inhibit) a Na+, K+-ATPase and/or ion porter (pump) system in the same membrane. Thus, only menadione, among the studied 1,4-naphthoquinones, interacts just with the receptor protein; and only its increasing repellency with increasing concentration is described by three distinct ($P<0.01$), but overlapping, linear regressions (Rozental & Norris, 1975; Norris, 1985, 1986). These three distinct regressions are compatible with the presence of three classes of menadione-binding sites in the receptor protein (Norris, 1985, 1986). The saturation of all three sets of such sites with menadione is represented in vitro by the maximum mV shift in receptor protein E 1/2 (i.e., 13-15 mV).

In the case of the more extensive studies with **P. americana**, including two behavioral biotypes (LAB and WARF), the input variable, (X) M_{99} = times (X) of moles of a given 1,4-naphthoquinone required in the assay tub to elicit greater then (>) 99% behavioral avoidance by the exposed cockroaches, is linearly related ($r^2 = 0.99$) to the measured primary output variable (mV Max = maximum mV E 1/2 shift induced in the neuronal protein by that 1,4-naphthoquinone). Further, both (X) M_{99} and mV Max are highly linearly related to the measured seconday output variable, max % Inh EAG = average maximum % inhibition in millivolts (mV) of the standardized EAG by the given 1,4-naphthoquinone. A computerized simultaneous solution of these three involved linear relationships showed that mV Max and max % Inh EAG are so highly correlated ($r^2 = 0.95$) that only one of them needs to be considered in an equation which expresses linearly the transduction of 1/4-naphthoquinone-molecular energy into whole-insect behavioral change (i.e., to avoidance). A resultant linear equation to express this transduction of chemically based energy into whole-insect behavior (avoidance) is here formulated as $\text{Log } Y = 3.40 - 0.112 (\text{Log } X)$, where Y equals mV Max and X = (X) M_{99}. The unit of measurements is mV.

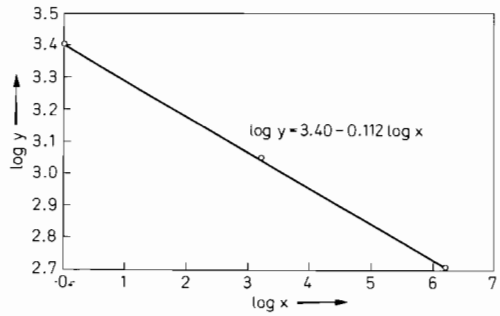

Figure 1. Linear relationship which exists between the primary input (afferent) variable, X (i.e., (X) M_{99}), and the primary output (efferent) variable, Y (i.e., mV Max), in **Periplaneta americana** perception and behavioral avoidance of 1,4-naphthoquinones as allelochemicals. In this situation certain 1,4-naphthoquinone-induced changes in the mV shift dictate behavioral alterations (e.g., non-avoidance to avoidance) in the whole insect.

To consider further the involved encoding of the transduced chemical energy into information, in the form of nerve impulses, let us examine the overall experimentally demonstrated situation with 2-methyl 1,4-naphthoquinone (menadione) because it only interacts with the redox-complex receptor protein in the dendritic membrane (Norris, 1985, 1986). Behaviorally obtained data with this repellent apparently indicate the existence of three distinct classes of menadione-binding receptor sites, designated A, B and C by Norris (1985). A saturation of all three sets of sites on this receptor protein is accompanied by > 99% whole-insect avoidance behavior. In vitro saturation of these three sets of site in LAB or WARF receptor protein with menadione is indicated electrochemically by a max mV E 1/2 shift of 13-15 mV. Our overall E 1/2 data on menadione-induced maximum shifts in the receptor protein support each shift as consisting of three increments, each being 4-5 mV (Norris, 1985, 1986). Thus, the menadione-induced 13-15 mV maximum E 1/2 shift in receptor protein of LAB or WARF seems to be composed of three, 4-5 mV, increments.

Because of the highly linear relationship between the primary output variable, mV Max, and the secondary output variable, max % Inh EAG, this latter variable for menadione, i.e., 23-25%, is here considered also as a multiple of three (3) equal increments. In such a consideration, 8% would be the obvious incremental value; thus, 3x8=24%. This leads us to the interpretation that a receptor E 1/2 shift of 4-5 mV equals a % Inh EAG value of 8%.

From the overall regression of the numbers of cockroaches behaviorally present upon the increasing concentration of applied menadione (Rozental & Norris, 1975; Norris, 1985, 1986), it appears that whole-cockroach behavioral change (i.e., avoidance vs. non-avoidance) occurs first while only receptor sites of Class A are being occupied. If so we might conclude that such initial behavioral change occurs in **P. americana** when the

corresponding % Inh EAG is not more than 8%; and the mV shift, not more than 5 mV. We may also conclude that the information required for such a whole-insect avoidance behavior by **P. americana** is initially encoded in the involved primary chemosensory neurons in the antenna. The CNS only must connect proper primary afferent neurons to proper efferent neurons attached to muscles involved in accomplishing the avoidance.

Our experimental results also apparently reveal two distinct levels in the evolution of this chemoreception mechanism in **P. americana** (Norris, 1985). Menadione saturation of all three (i.e., A, B and C) sets of binding sites in the involved receptor protein of each biotype involves a similar quantity of shifted electrochemical energy, i.e., 13-15 mV, in this polypeptide. Thus, this quantitative electrochemistry, which is apparently involved in generating nerve impulses, i.e., information, in the primary sensory neurons, is conserved (i.e., kept the same, 13-15 mV) in both biotypes. However, at the whole insect behavioral level, the LAB biotype is about twice (2X) as sensitive as the WARF to menadione as a repellent (i.e., 10^{-2} M for > 99% LAB behavioral avoidance vs. $2x10^{-2}$ M, for WARF). Thus, twice as much menadione repellent must be presented in the WARF's environment as in the LAB's in order for a given (similar) impulse-based neuronal message, dictating initiation of a whole-insect avoidance behavior, to be generated.

These above two distinct levels of evolution thus have allowed for the conservation (retention), in both biotypes, of an effective quantitative electrochemical (13-15 mV) information-encoding mechanism involving the receptor protein, while simultaneously enabling the development of biotypes (e.g., LAB and WARF) within the species that have distinctly different (2X) whole-insect behavioral sensitivities. The biochemical-biophysical mechanism for allowing such behavioral diversity, while retaining the evolved information-encoding device, is now under study, and seemingly involves some sensillum liquor-contained proteins which we have named "interfacins" (Norris, 1986). These proteins are physically situated between the dendrites of the primary chemosensory neurons in sensilla and the external environment, which may contain repellent chemicals. Interfacins bind repellent molecules, and thus influence the moles required to yield > 99% whole-insect behavioral avoidance (X) M_{99}. It seems very significant that the interfacins of WARF bind about twice (2X) as much menadione repellent as do those of LAB. This biotypical difference could easily account for such of their 2X difference in whole-insect behavioral sensitivity to this repellent and antifeedant.

Acknowledgements

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INTERACTION BETWEEN VISUAL AND OLFACTORY SIGNALS IN CONE RECOGNITION BY INSECT PESTS

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1. Introduction

Forty-one among the sixty insect species known as cone pests in Europe are host-specific. They generally damage cones of a single tree species or, at most, a few species belonging to a same genus (Roques, 1983). In most cases, host selection is realized by mature females that lay eggs on the cones during very specific and short-lived host development stages. Cone production often shows very irregular fluctuations from one year to another and among trees in the same stand. Success in egg-laying requires therefore the precision of a double synchronization, spatial and temporal, between females and cones.

Previous results lead us to assume that the naturally existing contrast in reflectance between cones and foliage of the larch could elicit positive orientation of a larch cone fly species (**Lasiomma melania** Ackl., Anthomyiidae) to cone-bearing trees (Roques, 1986). Correlations in several cone pests between the extent of cone crop damage and the terpene content of cones (Annila & Hiltunen, 1977; Oshkaev, 1981) tend also to imply olfactory signals emitted by the cone during its susceptible stage.

For these reasons, our objectives were i) to confirm the assumed role of reflectance contrast in **L. melania**'s behaviour, ii) to determine whether the role of visual signals is generalizable to other cone pests, according to spectral characteristics of their respective hosts, iii) to test the olfactory attraction of cone extracts taken at various stages of their development.

In addition to the larch cone fly, we focused on two other cone pests: the douglas-fir seed-chalcid, **Megastigmus spermotrophus** Wachtl., Torymidae, (monophagous, Hussey, 1955; Lessman, 1971) and the pine cone weevil, **Pissodes validirostris** Gyll., Curculionidae, (oligophagous damaging some pine cone species, Annila, 1975).

2. Results

Response of **Lasiomma** to colour combinations and shapes. Figure 1 details the results of a field trapping program performed in the French Alps using vertical sticky traps according to a previously described technique (Roques, 1986). In the first two tests (Exp. 1 and 2), very close colour combinations were compared for preference by both sexes. All were derived from the combination Bright Yellow with Purple stripes, which proved earlier to be the most attractive one for sexually mature flies. Each trap consisted of a square (20 cm x 20 cm) sheet of letraset'Pantone'coloured in

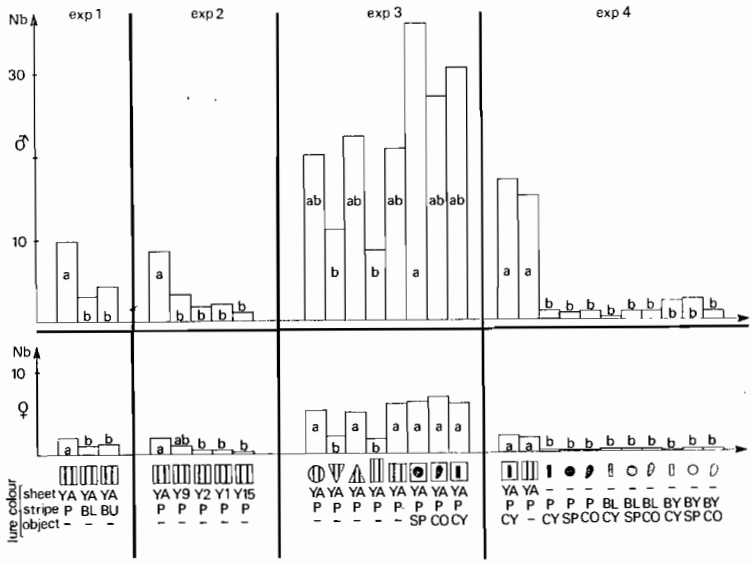

Figure 1. Mean number (Nb) of **Lasiomma** of each sex captured per week (3 replicates) in the French Alps by vertical sticky traps offering different colour combinations (exp. 1-2) and shapes (exp. 3-4); 'Pantone' colours used: YA=Yellow A (approximately Bright Yellow); Y1=J101 (app. Light Yellow); Y2=J102 (app. Lemon Yellow); Y9=J109 (app. Golden Yellow); Y15=J115 (app. Golden Yellow Light); P=Purple A; BU=Reflex blue; BL=Black. Objects: CY=Cylinders; SP=sphere; CO=cone. Any two columns with the same letter in the same experiment are not significantly different compared by a Newman and Keuls'test.

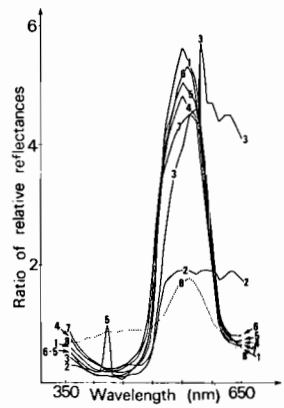

Figure 2. Variations in the ratio of relative reflectances (a) of the two elements of each trap used in the colour combination test (Fig. 1, exp 1): 1=YA vs P; 2=YA vs BU; 3=YA vs BL; 4=J101 vs P; 5=J102 vs P; 6=J109 vs P; 7=J115 vs P-(b) of larch foliage vs larch cones: 8. Values measured within the 350-650 nm wavelength range with a spectrophotometer Varyan Cary 17D, equipped with an integrating sphere.

Yellow, on which two stripes (20 cm x 1 cm) of dark shade were vertically glued at an equal distance from the edges. We compared three dark shades arranged with the same basic colour of the sheet (Bright Yellow- Exp. 1) and five hues of yellow, ranging from Light Yellow to Golden Yellow, combined with the same Purple stripes (Exp. 2). In both cases, traps coloured Bright Yellow with Purple stripes caught significantly more flies, males and females, than other traps did. More than 50% of the males and 75% of the females were mature. However, females were always trapped at least in a ratio 1:5 relative to males, whereas the natural sex-ratio is about 1. The number of flies captured per trap did not appear to be related to the ratio of total intensities of light reflected by each part of the trap from 350 to 650 nm (i.e., the ratio to total reflectance of the sheet to total reflectance of the stripes). Conversely, captures were positively correlated ($P<0.01$) with the ratio of relative reflectances of the two trap elements, observed from 535 to 555 nm (Fig. 2). This ratio reveals the contrast of purity between the element hues in the wavelength interval, the relative reflectance being the amount of light reflected within the interval relative to the total amount of reflected light (from 350 to 650 nm). Comparison of larch foliage and larch cone reflectance shows that the ratio of their relatively reflectances, foliage vs cones, peaks in the region 515-555 nm, too (Fig. 2).

In the shape tests (Fig. 1, exp. 3-4) two-dimensional plates of five geometrical shapes, though offering identical colour combinations (Bright Yellow with Purple stripes) and equal surface areas (400 cm^2), were compared among themselves and with three-dimensional traps. In a first test (exp. 3), these last traps consisted of the same yellow square sheet on which various plaster objects (cone, cylinder, sphere), coloured in purple, replace the stripes. In a second test (exp. 4), the same objects presenting various colours but the same total areas (150 cm^2) were directly suspended by wire from larch branches. Tree-dimensional lures showing colour combinations trapped generally more flies than two-dimensional ones did but significant differences were noticed only with two shapes (rectangle and inverted triangle) that appeared less attractive. The male/female ratio was unchanged from the previous results. Single objects caught significantly fewer flies compared to other traps.

Responses of **Megastigmus** and **Pissodes** to colours and colour combinations.

Laboratory tests with adults, directly emerged from cones collected in the field, were made un upright plastic cylinders (50 cm height x 100 cm diameter), derived from those used by Göttsche (1977) and Vandersar and Borden (1977). Response to ten different colours (Black, Dark Green, Dark Blue, Purple, Rose, Dark Red, Light Green, Bright Yellow, Golden Yellow, White) and five colour combinations (Bright Yellow x Purple, Light Green x Purple and Golden Yellow x Purple for **Megastigmus;** Bright Yellow x Black and Light Green x Black for **Pissodes**) were compared. Colours were presented as rectangular bands of 'Pantone' of equal dimensions (5.25 x 15 cm), vertically glued in a random order, with 3 replicates, on the wall of the arena. Each band was separated from the next by a space equal to its own width and the whole arena was uniformly illuminated by fluorescent lights

mounted 10 cm above. 200 Insects of each species were released at random in the centre of the arena, in groups of ten individuals of the same sex and similar development stage. Each insect was scored at the point where it first contacted the wall, even if this did not correspond to a coloured band (non-responding insects). The null hypothesis stating that insects randomly contact the periphery was rejected in all experiments, by comparison of responding and non-responding totals (χ^2 test, $P<0.01$).

However, the nature of the response appeared different for the two species. **Megastigmus** flew away after release and directly landed on the wall. Emergent individuals, both males as females, showed significant preference for Golden Yellow. This preference was lost in fed females for which the colour combinations Yellow and Green with Purple stripes appeared significantly more attractive. Fed males were not attracted to these last colours. In contrast, **Pissodes** contacted the periphery by walking and showed from emergence a continuous and quite-exclusive (80% of the choices) preference for dark shades.

A field trapping program using the colours tested in the arena was totally ineffective for these species. **Megastigmus** females, particularly, were observed on several occasions flying toward lures offering colour combinations, then avoiding the trap by a detour of about 20 cm in front of it, and finally landing directly onto cones located beneath the trap.

Comparative reflectance of foliage and cones of resinous trees. Conifers growing in Europe can be separated in two groups, regarding the difference between cone and foliage reflectance. In Scots pines, a cone in attractive stage (2nd year of development) and foliage of the corresponding year present quite parallel reflectance curves within the 500-600 nm range, and both peak at ca. 550 nm (Fig. 3a). Consequently the ratio of their relative reflectance (foliage vs cone) shows few variations with wavelength. Similar stable ratios characterize most trees with cones developing over several years: **Pinus halepensis, Pinus pinea, Pinus nigra, Cupressus sempervirens** and all European species of **Juniperus.** Conversely, peaks or reflectance are shifted in the 2nd group of species (Fig. 3b), with native foliage reflecting maximally between 540 and 560 nm whereas a cone reflectance peak occurs at about 630 nm. Thus, the ratio of their relative reflectances varies strongly from 350 to 650 nm with a maximum within the 525-560 nm range. Species of this group mostly possess mostly cones with annual development, (**Larix decidua, Pseudotsuga menziesii, Picea abies, Pinus cembra, Picea obovata, Abies koreana**). Exceptions in the two groups are **Abies alba** and **Cedrus atlantica** respectively.

Responses of **Lasiomma** and **Megastigmus** to cone extracts. Douglas-fir and Larch cone extracts were prepared for each of the four typical stages of the cone development (Roques, 1983, 1986): Bracts (B), Dominant Bracts with visible Scales (BS), Dominant Scales with still visible Bracts (SB), Scales (S). These extracts were made from 50 to 100 cones collected at the same development stage, on the same tree, and frozen for less than 6 months. After defrosting, cones were immersed in 250 cm^3 of pentane, the resulting solution being, then 5 times concentrated under reduced pressure (20 mn) at

room temperature.

Bioassays were performed in a tubular glass olfactometer (60 cm length x 2.5 cm diameter), using an experimental design similar to the one used by Lecomte and Thiboud (1981). Forty individuals of each sex of **Lasiomma** and **Megastigmus** were tested at emergence and after being fed for 7 days on a sugar solution. Each individual was exposed successively to pure air and to air-borne volatiles emitted from cone extracts (0.5 ml pipetted onto a filter paper strip), blown during one minute each at the same speed (20 cm/s). Tests were conducted between 11 am and 03 pm (time of maximal adult activity), at 21-22°C. In most cases, insects showed only locomotory activity, without flight. The single fed (mature) insects presented noticeable differences in response to cone volatiles. Comparison of insect distributions in the olfactometer at the end of each minute, with and without, volatiles revealed that mature females of each species were significantly attracted by the respective BS stage extracts (X^2 test, $P<0.05$). This stage corresponds in the two species to the one naturally selected for egg-laying. Males did not exhibit similar attraction. Activity of mature **Lasiomma** males was stimulated by three of the four large cone extracts whereas the single bract extracts released this effect in mating females. Locomotory activity of mature **Megastigmus** females proved also to be stimulated by BS extract whereas males were not, by any extract.

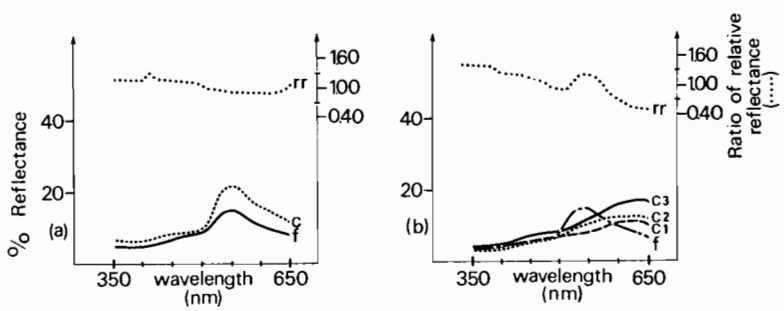

Figure 3. Spectral reflectance curves of foliage (f) and cones (c1, c2, c3), and variations in the ratio of their relative reflectance (rr=foliage vs cone) in two resinous species (a) **Pinus silvestris**; (b) **Pseudotsuga menziesii**; c1=B cone stage; c2=BS stage; c3= SB stage (see text).

Discussion-Conclusion

Visual and olfactory cues seem therefore clearly involved, but at various degrees according to host characteristics, in the recognition process of some cone species by their specific pests. Insects also appear attracted to cones only when mature. Significantly higher captures of sexually mature **Lasiomma** by lures that most amplify the natural reflectance contrast existing between larch cone and larch foliage confirm the presumed part of visual signals in this first species. The ratio of relative amounts of light, reflected by these structures within the 520-560 nm range, appears more important than the difference in total intensities of reflected light, contrary to our previous assumptions. The degree of

Lasiomma preference for colour combinations, being irrespective of shape, suggests that the most attractive lure might be a combination of a yellow colour, offering the greatest hue purity in this wavelength region (supernormal foliage-type stimulus), with a dark shade showing its lowest relative reflectance in the same range. **Megastigmus** response to visual lures seem to be of similar nature. Sugar nutrition is necessary to adult survival in laboratory and so the attractivity of Golden Yellow-coloured bands to emergent insects may correspond to a nutritional-type stimulus that elicits food-seeking behaviour, in the same way that horizontal yellow-coloured lures act for immature **Lasiomma** (Roques, 1986). **Megastigmus** emergence is effectively synchronized in Douglas-fir stands with the flowering broom (**Sarothamnus scoparius**), whose flowers are coloured Golden Yellow. However, we have never captured it on these flowers. Likewise, the existence of a distinct maximum, though attenuated in respect to larch, in the ratio of relative reflectance of Douglas-fir foliage vs Douglas-fir cones could explain modified reactions of mature **Megastigmus** females, searching for cones, to colour combinations offering a maximum in the similar wavelength range.

Conversely, **Pissodes** response to dark shades suggests a 'silhouette' effect. This fits with the weevil's behaviour. Emergent insects are generally observed feeding primarily on leader shoots before moving towards to cones. We have also to consider that the various hosts of **Pissodes** all belong to the first group of tree species defined above, presenting no relative reflectance contrast between foliage and cones.

Thus, it can be speculated that visual cues act as signals for specific pests when a contrast between relative amounts of light reflected by foliage and cone is noticeable within the wavelength region corresponding to foliage maximal reflectance. Some data indicate, nevertheless, that this signal plays a limited part in the female's decisive choice. Firstly, the contrast is not host-specific, being similar in most trees with annual cone development. It shows few variations with cone development, too, and cannot elicit the choice of a definite cone stage for egg-laying. Secondly, avoidance of traps in the field by mature **Megastigmus** females as well as limited proportion of **Lasiomma** females trapped in all experiments suggest that other factors influence short-distance cone discovery. Therefore, contrast of cones vs foliage may only allow pests to distinguish cone-bearing trees at a distance.

Oshkaev (1981) hypothetized that selection of both tree species and cone development stage would be controlled by volatile chemicals, emitted from cones in a specific blend during the susceptible stage. The observed attraction of mature females to the single extracts corresponding to a sensible stage agree with these assumptions, though care must be taken of experimental conditions, the insects having been tested when walking and not when flying. The stimulation effect of extracts on locomotory behaviour is also interesting in regard to natural behaviour of females of the two species. After landing on the cone, they move quickly in all directions until they find a suitable place for egg-laying (Hussey, 1955; Lessman, 1971; Roques, 1986). However, the absence of oviposition during the experiments strongly suggests and interplay of tactile stimuli in this

ultimate phase. Differences in male responses could be related to the location of mating, occurring on cones in **Lasiomma** and on shoots or needles in **Megastigmus** (Hussey, 1955). Which volatile chemicals are involved in olfactive signals? Oshkaev (1981) focused attention on monoterpene content and attributed larch cone attractivity to **Lasiomma** to a higher proportion of β-pinene. Both Annila and Hiltunen (1981) and Oshkaev (1981) considered that **Pissodes** attraction is related to α-pinene content of pine cones. A first raw chromatographic analysis of our extracts reveals significant changes in terpene content. The attractive Douglas-fir extract is characterized by both a maximal content of α-pinene and limonene and a minimal content of β-pinene and sabinene. The attractive larch cone extract presents no difference with other extracts in α-pinene and β-pinene, but a maximal content of limonene and an unindentified sesquiterpene.

In conclusion, it appears difficult to point out a single model for the selection strategy of cone pests. Olfactoy signals probably provide the specificity of choice in most cases. However, when cone visual characters are clearly distinct form those of the foliage in a tree species, it can be assumed, after Prokopy and Owens (1978) that specific pests associate a visual search image with the chemical attraction for locating cone bearing-trees.

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DYNAMICS OF HOST ODOR AND VISUAL STIMULUS INTERACTION IN HOST FINDING BEHAVIOR OF APPLE MAGGOT FLIES

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1. Introduction

In the realm of insect/plant interactions, considerable effort has been devoted to examining orientation behavior of insects toward plant olfactory stimuli (Finch, 1980; Visser, 1986; Payne et al., 1986; Robert, 1986). Less effort has been invested in examining insect orientation toward plant visual stimuli (Prokopy & Owens, 1983; Robert, 1986). Little effort has been directed toward examining the interplay of olfactory and visual stimuli during insect search for plant resources (Prokopy, 1986).

One insect that has received attention in regard to orientation to both host plant olfactory and host plant visual stimuli is the apple maggot fly (AMF), **Rhagoletis pomonella** (Walsh) (reviewed in Prokopy & Roitberg, 1984). Mature females respond positively from a distance (yet undetermined) to host fruit odor and tree visual stimuli. The blend of esters that comprises the fruit odor stimulus elicits, over time, accumulation of females at an upwind source in laboratory wind tunnels (Fein et al., 1982). Tree visual stimuli consist of green leaf color, silhouette of tree against background, tree size, and tree shape (compact). Unlike the host fruit odor blend, none of the visual characteristics is specific to host trees. After arrival on a fruiting host tree, females respond positively from short range (ca. 1 m or less, Roitberg, 1985) to fruit visual properties such as spherical shape, characteristic size, and contrast of color against background.

Until now, the focus of research on host finding in AMF has been largely on assessing responses of populations of AMF to plant odor stimuli alone or plant visual stimuli alone. Here, we describe briefly some of our recent research aimed at tracking inter-tree and intra-tree movement patterns of individual AMF females in semi-natureal settings that involve an interplay of fruit odor and plant visual stimuli. More detailed accounts will appear in future publications.

2. Materials and methods

All tests involved 13-19 day old mated females whose larvae developed in apples collected from nature. A few minutes prior to testing, each female was allowed to lay a single egg in an uninfested **Crataegus mollis** hawthorn fruit (= native host of AMF) to enhance the likelihood that test females were in a host-searching mode.

Inter-tree distance of response to host stimuli. These tests, conducted in an open grass field (80 x 200 m) completely surrounded by non-host trees, were designed to assess the distance at which AMF manifest a behavioral reaction tree models, with or without synthetic apple odor. AMF were released singly onto a lower leaf of a fruitless potted **C. toba** tree (1.5 m tall, 0.75 m^3 canopy volume) placed in the center of the field. The release tree was surrounded by 4 equi-distant 1 x 1 m square plywood tree models placed either 0.5, 2.5, or 4.5 m from the periphery of the tree canopy. All 4 models were painted entirely either with titanium oxide white (= control models mimicking skylight) or with the following mixture (by weight) of Winsor-Newton (London) artist oil pigments to closely mimic host leaf color (Owens, 1982): 83% cadmium yellow, 12% Winsor green, 5% mars black. Each model was perforated with 144 4-cm-diam holes to approximate patches of space between tree leaves and to permit air movement through the model. Two polyethylene vials (Reissing et al., 1985), both of which were either empty (= controls) or partially filled with the Fein et al. (1982) blend of synthetic apple volatiles (release rate = ca. 500 ug/hr, which equals that used in monitoring traps for AMF - Reissig et al., 1985) were attached at the lateral margins of each model. Each AMF was followed by 2 observers until it left the center tree, up to a maximum of 1 hr. The total time elapsed before departure, number of leaf visits, wind speed and direction, temperature, direction of take-off at time of fly departure and proportion of AMF alighting on a model were recorded.

Inter-tree movement pattern. To assess patterns of inter-tree movement, 25 fruitless potted **C. toba** trees (2.5 m tall, 0.9 m^3 canopy volume) were arranged in a 5 x 5 m grid on a flat piece of ground at the center of an open grass field (70 x 90 m). The peripheries of the tree canopies were 1 m apart. Polyethylene vials, either all empty (= controls) or all partially filled with the Fein et al. (1982) apple volatile blend (release rate = ca. 500 ug/hr) were attached 1.5 m above ground to metal poles (1 vial/pole) positioned every 2 m along the entire periphery of the grid, 2 m from tree canopy edges. AMF were relased singly onto a lower leaf of the center tree and were followed by 2 observers for 1 hr or until lost from view. The time spent in each tree, number of leaves visited/tree, number and location of trees visited, direction and speed of wind (recorded every 10 sec), temperature, and direction of take off at time of fly departure from a tree were recorded on tape.

Intra-tree response to fruit stimuli. In brief, the methodology for assessing intra-tree responses (described in detail elsewhere - Aluja et al., submitted) consisted of enclosing a fruitless apple tree (canopy diam. = 2.5 m) in a 3 1/2 x 3 1/2 m cylindrical screen cage. The tree was divided into ca. 3000 20 x 20 x 20 cm imaginary cubes of space. Every plant part falling within a particular cube was marked with a distinctive number, corresponding to X, Y and Z coordinates. Fruit-mimicking spheres or real fruit were hung from tree branches under no choice conditions. There were: (a) 3 densities of spheres or fruit (1, 4, or 16/tree); (b) 3 levels of the Fein et al. (1982) synthetic apple odor blend released in polyethylene

vials or plastic hollow fibers attached to the sphere (no odor; 0.7 ug/hr, which approximates the release rate of the volatiles from a single ripening Red Delicious apple; or 500 ug/hr); and (c) 3 levels of synthetic apple color (clear glass sphere, 8 cm diam; green rubber sphere, 6 cm diam, coated with a mixture of artist oil Winsor green (1.5%) and cadmium yellow (98.5%) (Winsor - Newton) to mimic the reflectance of a green Red Delicious apple; or red wooden sphere, 8 cm diam, coated with Tartar Red dark™ paint (Sherwin-Williams) to mimic the reflectance of a ripe Red Delicious apple. Real apple treatments consisted of green Red Delicious apples (6 cm diam) or ripe Red Delicious apples (8 cm diam). Each AMF, released singly from a lower leaf near the tree center, was tracked by an observer using a tape recorder as it moved from cube number to cube number until it left the tree or until 20 min elapsed. Data were recorded on: percent AMF discovering a sphere or real fruit; foraging time, total distance flown, and number of cubes visited before landing on first sphere or fruit; rate of movement (distance ÷ time); and turning angles during movement.

3. Results

Inter-tree distance of response to host stimuli. Among tree models without odor, results to date (Table 1) indicate AMF spent significantly less time on the release tree when green models were at 0.5 m than when green models were at 2.5 or 4.5 m or white models were at any of these 3 distances. There was no significant difference in fly response among green and white models at 2.5 and 4.5 m. Among tree models with synthetic apple odor, results to date (Table 1) indicate AMF spent significantly less time on the release tree when green models were at 0.5 and 2.5 m than when green models were at 4.5 m or white models were at 2.5 or 4.5 m. There was no difference in fly response when green vs. white models were at 4.5 m. The combined results suggest a trend toward less time spent on the release tree when either green or white models at 0.5 or 2.5 m (but not 4.5 m) were supplemented with apple odor than when without odor. Additional data (not presented) suggest that at moderate wind speeds (ca. 0.5-2.0 m/sec), AMF flew toward upwind models, irrespective of presence or absence of apple odor.

Table 1. Median time (min) spent by AMF on release tree when tree surrounded at different distances by 4 green or 4 white tree models (1 x 1 m) with or without synthetic apple odor (median test, 0.05 level, comparisons among all treatments) (N = 14/treatment).

Distance of model from tree (m)	No Odor		Odor	
	Green	White	Green	White
0.5	7.7a	16.2bc	6.2a	10.3ab
2.5	24.7c	23.8c	7.0a	17.6bc
4.5	15.2bc	19.0bc	23.5c	23.9c

Inter-tree movement pattern. Observations to date on the 14 AMF tracked within the patch of **C. toba** trees in the absence of host fruit odor and the 14 AMF tracked within the patch in the surrounding (hence continuous) presence of synthetic apple odor indicate that about 50% of inter-tree flights were upwind (especially at moderate wind speed), 30% downwind, and 20% crosswind. The tendency toward upwind flight was characteristic of flights in the absence as well as the presence of apple odor. The median number of trees visited by a fly before it was lost from view or 1 hr had expired was the same (4) for tests in the absence as well as the presence of apple odor. However, in the presence of apple odor, the median amount of time spent in each tree visited was significantly less (225 sec) than in the absence of odor (360 sec) (median test, 0.05 level).

Intra-tree response to fruit stimuli. A subset of the results (Table 2) indicates that at the 16 fruit/tree density level (500 ug/hr release rate of synthetic apple odor for treatments having odor), a significantly smaller percent of AMF arrived on odorless clear spheres before leaving the tree or before 20 min had expired than on clear spheres with odor. Percent arriving on odorless green spheres was no different form that on green spheres with odor, real green apples, or clear spheres with odor. Percent arriving on odorless red spheres was no different from that on red spheres with odor or real red apples, all of which received a significantly greater percent of arrivals than any of the other treatments. This same pattern of results generally characterized AMF arrivals on clear, green, and red spheres at the 1 sphere/tree density level, both at a 500 ug/hr and at a 0.7 ug/hr release rate of odor. The only exception was lack of any difference in percent arriving on clear spheres with vs. without odor.

Table 2. Percent of released AMF alighting on a clear or colored fruit-mimicking sphere with or without synthetic apple odor or on a real apple (no-choice tests) hung within release tree (G test, 0.05 level, comparisons within-row only) (N = 25/treatment).

Sphere (fruit) density	Release rate of odor	Clear		Green			Red		
		No Odor	+ Odor	No Odor	+ Odor	Real Apple	No Odor	+ Odor	Real Apple
16	500 ug/hr	12a	36b	32ab	40b	36b	92c	88c	88c
1	500 ug/hr	0a	0a	12a	4a	--	48b	52b	--
1	0.7 ug/hr	0a	4a	0a	8a	--	52b	52bd	--

4. Conclusions

The observations and data reported here, coupled with unreported observations and data, lead us to draw the following tentative conclusions about the nature of AMF female response to host fruit odor and plant visual stimuli. When seeking fruiting host plants for egglaying, AMF tent to move upwind, especially under moderate wind speed conditions (e.g., 0.5 - 2.0

m/sec), irrespective of the presence or absence of host fruit odor in the area (cf. Finch & Skinner, 1982). However, in the presence of host fruit odor, AMF appear to move among trees at a faster rate (possibly enhancing the probability of ultimately encountering a tree with fruit) than when host fruit odor is absent. Analogous behavior might apply to AMF moving among non- or poorly-fruiting branches of large host trees that have some well-fruiting branches. Both host fruit odor and tree visual stimuli appear to stimulate a greater degree of positive response from AMF when closeby (e.g., 0.5 - 2.5 m) then when farther away (e.g., 4.5 m), with these stimuli seemingly acting in a synergistic fashion. The combined evidence suggests that after arrival on a small host tree, AMF find individual fruit, be they of green color (weakly visually stimulating) or red color (strongly visually stimulating) primarily or solely on the basis of fruit visual characteristics, irrespective of fruit density or strenght of olfactory stimulus. It should not be surprising that AMF use visual rather than olfactory information to locate individual fruit within the canopy of a small tree or within branches of a larger tree because wind- and foliar-shearing effects on the structure of odor plumes from numerous point sources of odor release (i.e. numerous fruit/tree or branches) would undoubtedly render it very difficult to track readily a single plume to its source. Additional research currently conducted by A.L. Averill, W.L. Roelofs. and W.H. Reissig at Geneva, New York and in our own laboratory should eventually give rise to a more comprehensive picture of the dynamics of host odor and visual stimulus interaction in AMF.

Acknowledgements

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FIELD RESPONSES OF MEDITERRANEAN FRUIT FLIES TO COLORED SPHERES SUSPENDED IN FIG, CITRUS AND OLIVE TREES

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1. Introduction

The Mediterranean fruit fly **Ceratitis capitata** (Wiedemann) (Diptera, Tephritidae), is an extremely polyphagous species ovipositing into the ripening fruits of more than 250 tree and vegetable species. As other fruit flies, **C. capitata** utilises chemical as well as visual cues in locating and selecting potential host plants and their fruits. Among visual cues, fruit shape, size and color are of great importance in fruit location and selection (Prokopy, 1977 and ref. therein). Consequently, field assessment of fruit fly responses to objects mimicking real fruits such as spherical ones, is of particular interest for the investigation of host fruit location and selection mechanisms in these flies.

Here, I report, part of an investigation on the field responses of a wild population of **C. capitata** to colored spheres suspended in fruiting host (fig, citrus) and nonhost (olive) trees, on the island of Chios, Greece. Some related information has been reported also from Hawaii by Nagakawa et al. (1978) working in a coffee plantation and from Brasil by Cytrynowicz et al. (1982), working in peach trees. For reasons of brevity, only responses to colored spheres of 7 cm diam. will be considered here and will be analysed in relation to the motivation of the flies. Further details and an analysis of the observed responses in relation to the color properties and to the size of the spheres will be presented elsewhere.

2. Materials and methods

The experiments were made between August 6th and September 20th, 1985 on the island of Chios, Greece, in the eastern Aegian Sea, on a ca. 4 ha farm producing mainly citrus fruits and vegetables. In and around the cultivated area there were also other tree species including fig and olive trees. The spheres tested were plastic, 7 cm diam., painted with seven different enamels: black, red, orange (Iliolac) and white (Super Royal Marine) from Ilios AG., (Psaron & Socratus, Piraeus, Greece), and green no. 48, blue no. 58 and yellow no. 24 from HELIOCHROM AG., (Moschato, Pireaus, Greece). The prepared spheres were covered with ca. 1 mm of adhesive "Tangletrap" (The Tanglefoot Co., Grand Rapids, Michigan, U.S.A.) to capture alighting flies. For the experiments, two large fig trees, two orange, two bitter-orange and one olive tree were used. The fig trees were ca. 8 m high, 10-15 m in canopy diam., ca. 100 m apart, and bore large number of fruits which were in all stages of maturity during most of the experimental period. The citrus trees were ca. 3 m high, 3 m canopy diam.

an bore unripe and ripening fruits from a last year's out-of-season blossom. Most of the ripening and ripe fruits of the fig and citrus trees were highly infested by **C. capitata**. Finally, a small, ca. 4 m high and 3 m canopy diam. isolated olive tree was used, located in an open field, ca. 10 m from the nearest citrus plantation and 100-200 m from the fig trees. It bore a moderate number of unripe, green olives.

In all test trees the spheres of all seven colors (= one set), were suspended from a wire, usually 30-50 cm apart, along a string stretched between two branches of a tree, 1.5 - 2 m above and parallel to the ground, 0.5 - 1 m from the outermost foliage. All foliage and fruits within a distance of ca. 30 cm to 1 m from the spheres was removed. Spheres in each set were randomly assigned. One set of spheres was used per tree. On most days, the captured **C. capitata** flies and other insects were removed from the spheres and counted daily, while on days of high captures, or when specific observations were conducted, flies were removed and counted 4-10 times a day. Additional adhesive was applied to the spheres as needed and their position was rerandomized within a set after every count. All spheres were replaced by new ones at least once a week.

To explore the motivation of responding flies, direct observations of the fly activities were conducted throughout the day. In addtion, the response of the flies responding to spheres suspended in fig and olive trees was monitored every 2-3 hours, or every 30-40 min in the hours of intensive fly activity, from morning until darkness, for 3-4 successive days. In each check, the captured flies were removed and counted and the position of the spheres rerandomized. In one case, for a 4-day period, also the response to 4 sticky-coated ripening figs located in one tree near the spheres was monitored.

3. Results and discussion

Throughout the experimental period, 9758 **C. capitata** flies were captured on all spheres, 6208 of them in the two fig trees, 1053 on the four citrus trees and 2497 in the olive tree (Table 1). It is apparent that on the host trees, the flies were strongly attracted by yellow spheres followed by orange, red, black and green which were almost equally attractive. The same preference was found for spheres suspended in the olive tree, except that yellow on that tree was equally attractive to the other four attractive colors. In all tree types white and blue spheres were the least attractive, except in citrus where white spheres captured as many flies as green. If captures in all tree species are considered, about 26% of flies were captures on yellow, 13-17% on orange, green red and black, and 5-8% on white and blue spheres. No essential differences were observed between males and females. On spheres of all colors and in all trees the number of attracted females was 2.5 times that of males. In total 1756 females were dissected of which 1466, i.e. 83.5% had mature eggs. No differences in the percentage of mature females were found between the different colors or the tree species in which the spheres were hung.

The response of flies during the different hours of the day for a period in September is shown in Fig. 1. Similar results had been obtained also earlier in the experimental period. As shown in the figure, the

attraction to the spheres suspended in the fig and olive trees was very strong during late afternoon, 1-2 hours before sunset, and continued also after sunset until dark. Captures on spheres were strongly correlated with observed fly visits to fruits, and to captures on sticky-coated figs. Intensive fruit visiting by the flies was related to observed intensive feeding on ripening, and mostly, on ripe and overripe fruits. During late afternoon and dusk, but not during morning and early afternoon. It was common to observe large numbers of **C. capitata** flies (and other insects) aggregating on these fruits for feeding, while oviposition activity was only low to moderate during that time. Up to 17 **C. capitata** flies were counted feeding simultaneously on a single ripe fig, while aggregations of 5-10 flies per fig were frequently observed, especially during September when the population was high (Fig. 2). It is concluded that the observed response of **C. capitata** flies to colored spheres, especially to yellow ones which were the most attractive, was predominantly a food-seeking response to ripe fruit-type stimuli and not a response to oviposition sites as could have been concluded by the fact that most of captured females were mature.

Table 1. Captures of **C. capitata** flies on sticky-coated 7 cm diam. spheres of seven different colors suspended on fig, citrus and olive trees in Chios, Greece between August 6 and September 20, 1985. Each replicate consisted of 2-4 day captures by a set of seven spheres, one of each color.

Color of spheres	Number of captured flies per color as % of total[1]			
	Fig trees (N=26)	Citrus trees (N=10)	Olive tree (N=10)	All combined (N=46)
Yellow	28.0 a	29.2 a	19.7 a	26.0 a
Orange	15.5 b	17.0 ab	19.6 a	16.7 b
Red	15.9 b	13.3 ab	18.9 a	16.4 b
Black	15.4 b	13.1 ab	14.4 ab	14.9 b
Green	12.1 b	10.5 bc	16.0 a	12.9 b
White	8.0 c	11.4 bc	7.0 b	8.1 c
Blue	5.3 c	5.4 c	4.4 b	5.0 c
Total captured	6208	1053	2497	9758
Females (%)	67.3	86.8	75.0	71.4

[1]Values in each column followed by the same letter are not significantly different at the 0.05 level (Wilcoxon-Wilcox test).

Although the feeding activity of the flies on figs was correlated to their visual response to the spheres, it was obvious that the flies observed feeding on figs, especially on overripe, decaying ones, which usually had lost their particular fruit shape, were attracted and arrested there not only by visual but also, or even mostly, by olfactory stimuli, emmitted by these fruits. On the other hand, visual stimuli were apparently also involved in the location and selection of the fruits as it is evident from the response of flies to the sticky-coated, and thus odorless, real fruits. On whether olfactory and visual stimuli act independently or interact by this process of fruit location for feeding, cannot be concluded

on the basis of the present results. The fact that sticky-coated spheres, especially the yellow ones, captured much more flies than the nearby located sticky-coated figs, indicates that yellow spheres were much more visually attractive than figs and thus, at least on these trees, they might constitute for the flies a super-normal ripe fruit-type stimulus on which to found food.

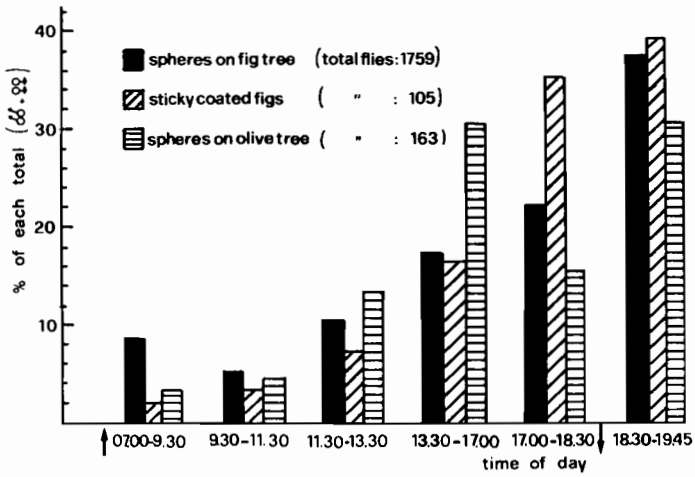

Figure 1. Number of **C. capitata** flies captured during different hours of the day on two sets of spheres (of seven different colors) suspended in a fig and an olive tree, and on four sticky-coated figs. Captures of a 4-day period (September 7-10) given as percentages of the mean daily captures. Time of sunrise (↑) and sunset (↓) is also given.

The present findings and interpretations differ in certain respects from those reported by other investigators (Nagakawa et al., 1978; Cytrynowicz et al., 1982). They differ in the order of color preference for spheres, and also in the suggested motivation of the responding flies. A number of reasons might be responsible for these differences such as the genetical make-up of the populations, originating from distant countries, the different host plants in which the spheres were suspended, the season, the population density, the number of the colors compared and possible differences in their spectral characteristics, the experimental design including the modus of sphere hanging in the trees and the frequency of counts and removal of captured insects. The color preference found in the present study is based on a large number of captured flies on the spheres tested and behavioral observations are made supporting the conclusions concerning the motivation of responding flies. However, it is evident that field data obtained under different ecological situations and locations should be compared with caution. Obviously, accumulation of differing data obtained under various situations might help to form a more clear picture of the basic behavior of the species and its possible variability.

Figure 2. Aggregation of **C. capitata** flies feeding on an overripe fig. Photo taken on September 14, a few minutes before sunset.

Abstract

The response of wild Mediterranean fruit flies, **Ceratitis capitata** (Wiedemann), Diptera, Tephritidae, to sticky-coated, 7.0 cm diam. spheres of seven different colors suspended in fruiting host (fig, citrus) and nonhost (olive) trees, was assessed during August and September 1985 in the island of Chios, Greece. In fig trees, yellow spheres were the most attractive, followed by orange, red, black and green, while white and blue were the least attractive colors. Almost the same preference was found for spheres suspended in citrus trees and for spheres suspended in an isolated nonhost tree (olive), except that yellow on that tree was equally preferred to the other four attractive colors. On spheres of all colors the number of attracted females was 2.5 times that of males and about 15% of the females were mature. The response to the spheres was very strong during late afternoon and at dusk and was highly correlated with observed feeding activity on ripe fruits and to captures on sticky-coated real fruits (figs). It was concluded that the response to colored spheres especially to yellow ones, was predominantly a food-seeking response to ripe fruit-type stimuli rather than a response to oviposition site-type stimuli.

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CHAPTER 4. INFLUENCE OF THE PHYSIOLOGICAL STATE, DEVELOPMENT AND BIOLOGICAL RHYTHMS OF PLANTS AND INSECTS ON THEIR INTERACTIONS.

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INFLUENCE OF BIOLOGICAL RHYTHMS, TISSUE DEVELOPMENT, AND PHYSIOLOGICAL STATE OF PLANTS AND INSECTS ON THEIR INTERACTIONS

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1. Introduction

Little information is available on tissue developmental changes in secondary products of host plants and the effect these changes may have on the windows of attack that are observed for herbivores. More information is available on the effect of the physiological state of host plants on herbivore dynamics although much of the data are conflicting. Research in this area has been on primary nutrition, particularly the response of herbivores to nitrogen variation in stressed plants. In this paper data on the changes in secondary products over time due to developmental or maturation processes within a growing season are presented. In addition, stress-induced changes in the physiological state of plants with regard to primary nutrition and secondary metabolites, and the affect of these changes on herbivores, are discussed.

2. Development changes in secondary products

Studies delineating quantitative changes in individual secondary metabolites during tissue development indicate that a great deal of variation exists. Generally, the pattern suggested by the literature is that younger leaf tissues contain higher concentrations of the toxic or qualitative secondary metabolites while mature leaf tissues were often shown to be lower (Feeny, 1976; Rhoades & cates, 1976). In addition, the digestibility-reducing or quantitative group of secondary metabolites were thought to be lower in the rapidly growing younger leaves and to increase substantially in the mature leaf tissues.

Studies tracking individual compounds over shorter time periods during the development of current needle tissue of Douglas-fir from bud to a year after budbreak indicate that a great deal of variation in the production of these compounds exists (Fig. 1). Data in figure 1 are modified from the original publications and represent only generalized patterns based on detailed studies of changes in the content of 14 terpenoids (in original publication data are expressed as mg/g fresh weight) over a 6-week period (Gambliel & Cates, 1986), and 6 individual phenolics, several unidentified phenolics (both expressed as peak area counts/mg dry weight), and astringency (% dry weight tannic acid equivalents) over a 52-week period (Horner, 1984). Only certain compounds are depicted in figure 1 to indicate some of the patterns that may be present; the reader is referred to the above references for detailes accounts.

Generally, at the initial time of budbreak terpene content is low and increases as the tissue develops. Some increases are dramatic such as those for bornyl acetate and camphene. Developmental change in the content of other terpenes may be gradual as represented by myrcene resulting in little significant increase over a 6-week period (at least for the plants from which tissue was analyzed). Still other, such as some of the sesquiterpenes, decrease in concentration during the maturation process (Gambliel & Cates, 1986).

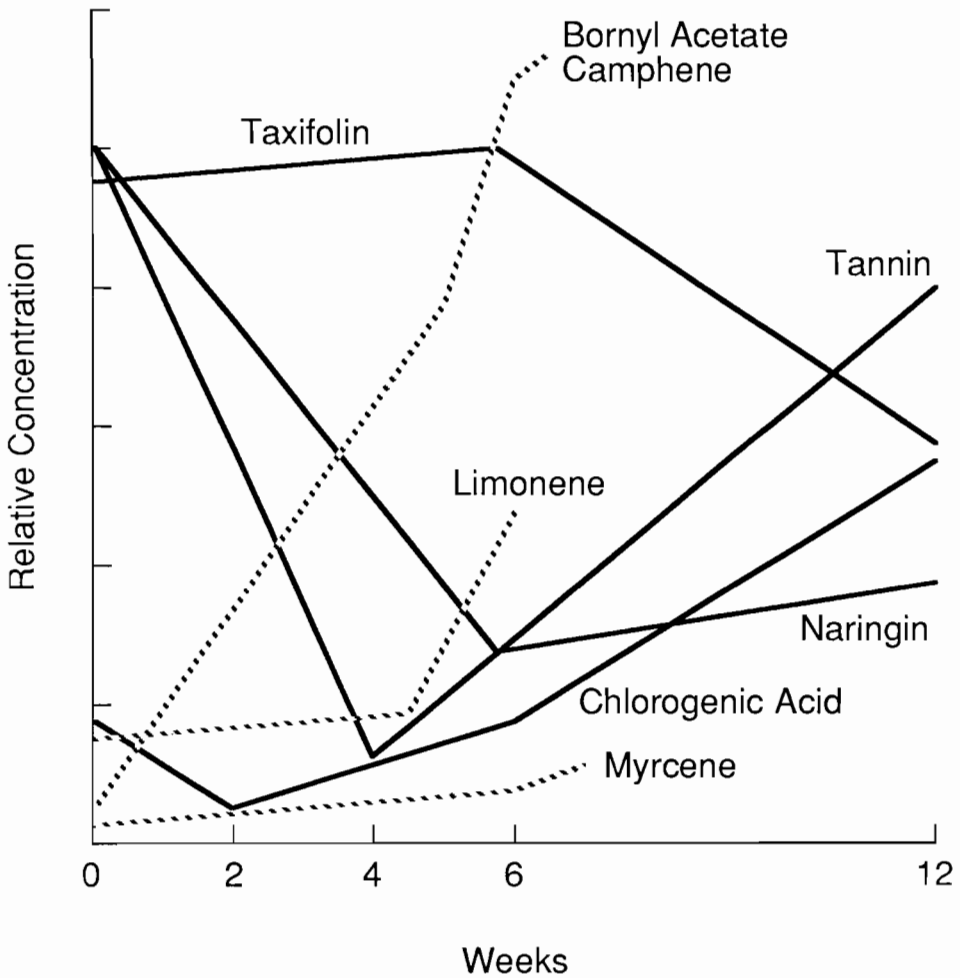

Figure 1. Developmental variation in the production of terpenes (broken lines) and phenolics (solid lines) in the current year's tissue of Douglas-fir. Terpene patterns are modified from Gambliel and Cates (1986); phenolic patterns from Horner (1984).

In contrast, phenolics show a different pattern in that some were found in high concentration in swelling buds (Horner, 1984). The magnitude of difference between terpenes and phenolics at time zero (and throughout tissue development in some cases) is in part due to terpenes being expressed on a fresh-weight basis and phenolics on a dry-weight basis in the original publications. Even when this is accounted for it appears that there may be a higher content of some phenolics in the swelling bud as compared to terpenes. A major point is that not all compounds follow the same pattern during leaf tissue development. Finally, although not depicted in figure 1, nitrogen content decreases during needle development, a pattern generally true of the leaf maturation process (Mattson, 1980).

A pattern of decrease in tannin content of young fronds followed by an increase has been observed for bracken fern (Tempel, 1981), while tannins of oak leaves gradually increase during leaf development (Feeny, 1970). Chlorogenic acid content of tobacco leaves (Sheen, 1969) and Douglas-fir needles (Radwan, 1975) was found to decrease and then increase during maturation. In leaves of **Heteromeles arbutifolia** phenolic and tannin content overall tends to increase during the growing season (Dement & Mooney, 1974). In contrast, sesquiterpenes in **Hymenaea** were found to initially be at high levels and then decrease with leaf age, while total phenolics, tannins, and astringency all decreased with leaf maturation (Crankshaw & Langenheim, 1981).

Developmental variation in the production of secondary metabolites, along with the usually decreasing primary nutrition (particularly nitrogen) during the maturation process of tissues, represent important factors in the evolution of the biological rhythms observed between plant tissues and the herbivores that exploit those tissues throughout the growing season. Resulting in part from this progressive qualitative and quantitative change in the primary and secondary metabolite content of plant tissues are the windows of attack observed in the herbivore community on leaf, root, stem, flower, and fruiting tissues.

For example, the western spruce budworm (**Choristoneura occidentalis**) feeds primarily on the developing current year's needles during the 4-5 weeks following budbreak (although larvae mine foliage buds and mature needles, for various reasons these are not the preferred tissues flower buds may be the most preferred of all tissues). It appears, based on studies delineating the secondary metabolite content of developing shoots (Horner, 1984; Gambliel & Cates, 1986) anb from figure 1, that the budworm may encounter the least qualitative and quantitative secondary metabolite chemistry by feeding on the current needles as soon after budbreak as possible. In fact, mining and feeding on the embryonic foliage in swelling buds may have been favored by natural selection as a mechanism to reduce exposure to the rapidly developing secondary metabolite chemistry as well as in avoiding the whims of the early season physical environment.

However, this scenario is not necessarily representative of the seasonal development in the secondary metabolites among all Douglas-fir trees within a population. Field studies utilizing similar-aged individuals from 4 populations of Douglas-fir have revealed that several terpenoids are correlated with decreased larval survival, reduced male and female biomass

production, and decreased fecundity (Cates & Redak, 1986, Cates et al., 1983; Redak & Cates, 1984). Some trees within a population are capable of producing appropriate quantities of certain individual terpenes or combinations of terpenes that adversely affect budworm success as measured by the above parameters. Other trees lack the ability, for one reason or another, to produce the deterrent compounds and are highly susceptible to budworm attack. In addition, McDonald (1981) has shown using controlled greenhouse studies that the 'resistance factor (s)' can be highly heritable in the current year's foliage of progeny of 'resistant' trees, while progeny of susceptible trees also appear to be susceptible.

In summary, utilizing examples from carbon-based secondary metabolites, it is clear that a great deal of variation exists in the production of secondary metabolites during foliage development. These developmental patterns, heavily molded in the genetic framework of plants ultimately by abiotic factors, form at least part of the basis for the biological rhythms that are observed between plants and the insects that utilize them.

3. Physiological state of plants and its affect on insects

Significant variation in the biological rhythms of plants can result often randomly by various 'stresses', such as drought, high temperatures, pollution, water-logging, fertilization, crowding, and others, resulting in the disruptment of normal developmental patterns and metabolic processes. Stress is not easily defined although most agree that the result of short-duration stress is a change in the physiological and metabolic state of plant tissues (Timmermann et al., 1984).

The consequence of these changes to plants and their associated insects and microbes is only one of many areas in the realm of stress physiology of plants that is in need of research (Gershenzon, 1984). For example, White (1969, 1974) suggested that increased water deficits resulted in physiological stress of host plants, that this physiological stress increased the amount of nitrogeneous food available to herbivores, and that the result was an increase in larval survival and adult reproduction that eventually could lead to outbreaks.

Table 1. Average number of larvae surviving and female adult biomass production (mg dry weight) per Douglas-fir tree. (Modified from Cates et al., 1983).

Site	No. larvae surviving	Biomass
Nonstressed trees	3.0	18.2
Stressed trees	5.2	23.6

In support of this, spruce budworm larvae that were reared on the current year's needles of trenched Douglas-fir trees survived at a significantly higher level and female biomass production was significantly greater as compared to larvae and females reared on the foliage of nontrenched trees (Table 1 and Cates et al., 1983). However, significant

differences in the terpene content of foliage from stressed trees also was observed in that all terpene content decreased except for alpha-pinene. Particularly noted was the decrease in bornyl acetate, which has been shown to significantly increase larval mortality and reduce adult biomass production and fecundity, in the foliage of stressed trees (Cates, Redak, Henderson, unpublished). It is important to note that the major changes in foliage quality in this study was not in the nitrogen content but was in the deterrent or toxic compounds like bornyl acetate and possibly other toxic terpenes. However, in a similar study in which Douglas-fir trees also were trenched and budworm were reared on trenched as well as the control or nontrenched trees, the results in part were the opposite. When budworm weights, obtained from rearing larvae on trees from each of 4 different groups, were subjected to discriminant analysis, it was found that only the budworm reared on the trenched trees that were growing on the south-facing slope were significantly ($P<0.05$) different in biomass production (Table 2). However, the difference was not in the direction of greater budworm weights from larvae reared on the trenched trees, as reported in the previous study (Table 1). The adult weights of budworm reared on the south-facing slope trenched trees were actually less than were the weights of adults from larvae reared on the nontrenched south-facing slope trees as well as less than those from larvae reared on the trenched canyon bottom trees and the nontrenched canyon bottom trees. A major difference between the two studies was that the effect of the treatment was greater in the study reported in table 1 as compared to that reported in table 2. The reasons for the difference in treatment affect on the physiological state of the foliage was that although both studies involved trenching, the former included partial root trenching near the tree bole while in the latter study trenching was at the canopy dripline (Cates et al., 1983; Cates & Redak, 1986). Xylem pressure potentials of the trees were more negative and the increased water stress in the season for the study reported in table 1 occurred earlier in the season than for the study reported in table 2 (xylem pressure potentials for the second study were reported by Horner, 1984). It appears that the canyon bottom trees that were trenched did not incur a significantly different water stress because capillary action from the water table at the canyon bottom site ameliorated the trenching effect.

Table 2. Discriminant analysis of budworm biomass production from larvae reared on each of the four sets of trees. (Modified from Cates & Redak, 1986).

Treatment	Discriminant scores
South-facing slope, trenched trees	-0.95
South-facing slope, nontrenched trees	0.05
Canyon bottom, trenched trees	0.06
Canyon bottom, nontrenched trees	0.37

When subjected to discriminant analysis to determine if the terpene chemistry was the same for all 4 groups, it was found that the chemistry of the south-facing slope, trenched trees was the only chemistry to be significantly different. However, as also noted for the xylem pressure potentials, the change in terpene chemistry for the study reported in table 2 was not as great as were the changes observed in the study reported in table 1 (Cates et al., 1983; Cates & Redak, 1986; Horner, 1984). Similar results were obtained by McCullough and Wagner (1986) in that **Neodiprion autumnalis** larvae, when reared on **Pinus ponderosa** trees that had been trenched, had lower pupal weights, lower survival, and longer feeding periods than larvae reared on nontrenched trees.

4. Conclusions

The patterns in the production of secondary metabolites during the development and maturation process of the current year's needles support the contention that the budworm is not extremely well adapted to the vicissitudes of terpene (and possibly phenolic) chemistry that are found in the needles among Douglas-fir trees in a population. These data suggest strongly that small variations in the phenology of both primary and secondary chemistry, as well as variation in budbreak phenology, could have important consequences to the fitness of budworm. Other field and laboratory studies are consistent with this idea (Cates et al., 1983; Cates & Redak, 1986; Redak & Cates, 1984). A greater understanding of the effect of variation in the production of secondary metabolites in the tissues of host species on the western spruce budworm may provide information that might increase the utility of silvicultural practices as mechanisms of control of the budworm.

These studies indicate that: 1) the intensity of stress can have an important influcence on the magnitude of foliage quality changes which in turn may influcence differentially herbivore response; and 2) that changes in secondary metabolites may be as important, if not more so, as are the changes observed in primary nutrition (Rhoades, 1976; Cates et al., 1983). As indicated by Gershenzon (1984) the intensity, duration, and type of stress may result in different outcomes in the physiological state of the plant which may in turn differentially influcence insect response. A great deal more information is needed concerning the patterns in the production of secondary metabolites in the normal tissue developmental and metabolic processes of annual, herbaceous perennial, and woody perennial plants. This along with investigations of the effects of stress on developmental and metabolic processes will be important in our understanding of the effects of stress on plant systems and secondary metabolites, and the responses of natural enemies such as herbivores and microbes to changes in the physiological state of plants.

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INFLUENCE OF THE INFLORESCENCE AND PODS OF VIGNA UNGUICULATA WALP (PHASEOLINAE) ON THE TERMINATION OF THE REPRODUCTIVE DIAPAUSE OF BRUCHIDIUS ATROLINEATUS (PIC) COLEOPTERA BRUCHIDAE.

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1. Introduction

Bruchidius atrolineatus is a tropical Bruchidae that is widely distributed in the Sahelian region. Wild and cultivated varieties of cowpea (**Vigna unguiculata**) is its principal host plant (Decelle, 1981). Studies in Niger show that adults of **B. atrolineatus** appear in the field at the end of the rainy season (mid August when **V. unguiculata** begin to flower) and reproduce on the young pods as soon as they are formed. Two generations occur, one during and one after ripening of cowpea pods (Huignard et al., 1985), most adults of the latter generation are in reproductive diapause (Huignard et al., 1983). This diapause is induced during the post embryonic development and is mainly dependant on the prevailing thermoperiodic conditions (Huignard et al., 1984). These diapausing insects probably take refuge in protected sites during the dry season and the beginning of the rainy season (5 to 6 months) until the flowering of the host plant. In tropical insects as in insects from temperate zones variations in photoperiod and thermoperiod influence induction or termination of diapause (Denlinger, 1986). The wide variations in relative humidity at the beginning of the rainy season could be a signal for diapause termination (Denlinger, 1986). In phytophagous insects stimuli coming from the host plant or from certain specific organs (leaves, flowers, fruits) induce sexual maturation (Carlisle et al., 1965; Pajni & Sood, 1975; Pescho & Van Houten, 1982). This study analyses the influence of host plant factors on diapause reproductive termination of **B. atrolineatus**.

2. Materials and methods

1) Insect rearing conditions. The strain of **B. atrolineatus** used in this experiment was from the Niamey region (Niger) and was raised in the laboratory on **Vigna unguiculata** seeds (variety TN 88/63). The females oviposited on the seeds and post-embryonic development took place in the seeds. When developmental conditions are 12 h L 40°Thermophase / 12 h D 25°Cryophase 60-70% RH, 85 to 90% of the adults emerging from the seeds after 25 to 40 days are sexually active. However, under conditions of 12 h L / 12 h D, 7 h 40°T. / 17 h 25°C., 60-70% RH, develoment time is much longer (from 55 to 85 days) but 85% of the emerging adults are in reproductive diapause (Huignard et al., 1984). To distinguish diapausing and non-diapausing insects, the insects were placed by couples in Petri dishes in the presence of **V. unguiculata** seeds for 10 days (Germain et al., 1985). If there was no oviposition after 10 days, the insects were

considered to be in diapause.

The insects in diapause were kept in the Petri dishes under developmental conditions (12 h L 40°T. / 12 h D 25°C.) for 3 months; they were fed pollen (collected by bees) and 10% sucrose water solution.

2) Experimental conditions. The diapausing bruchids were placed in groups of either 10 males or 10 females in cylindrical containers (d = 90 mm, h = 130 mm), with screened openings with or without vegetal organs of **V. unguiculata** (inflorescences, green young pods at differenct stages of maturity, dry pods, leaves). The experiments took place under climatic conditions similar to those observed in Niger at the end of the rainy season, 12 h L at 35°T. / 12 h D at 25°C., 70 to 90-100% RH. Insects in the experiment were dissected 10 and 20 days after the introduction of inflorescences or pods and the state of their reproductive organs noted.

For histological studies reproductive organs were fixed with Halmi and sections were stained with P.A.S..

3. Results

Characterization of the reproductive diapause. The diapausing adults have a high locomotor activity but no sexual behaviour. The females did not have vitellogenin in their hemolymph and the ovarioles were reduced to the germarium. They produced no sexual pheromones (Nammour, personal communication).

The emerging males have small testis containing spermatogonia and spermatids. During the imaginal life spermatogenesis was very slow and few spermatodesms appeared in the seminal vesicles. The male accessory glands showed no secretory activity (Germain et al., 1985). The diapausing males were insensitive or only slightly sensitive to sexual pheromone.

Influence of **V. unquiculata** inflorescences or seed pods on the termination of the reproductive diapause of **B. atrolineatus** (Table 1). When 90-day old diapausing males and females were placed in the presence of **V. unguiculata** inflorescences or green young pods, development of the reproductive organs was observed as early as the 10th day in most of the insects tested. In the females, vitellogenin was synthesized in the hemolymph and mature oocytes appeared in the ovarioles, then in the lateral oviducts. In the males there was a sudden acceleration of spermatogenesis and the spermatozoa accumulated in the seminal vesicles. The epithelium of the male accessory glands thickened and secretions appeared in the lumen. However, in the presence of only dry **V. unguiculata** pods or cowpea leaves, there was no development of the reproductive organs.

When the insects were dissected 20 days after the beginning of the experiment, the reproductive organs were developed in the majority of the bruchids placed in the presence of inflorescences or young pods, whereas the leaves and dry pods still showed no influence.

Reproductive activity of post diapausing insects (Table 2). It is possible that the stimuli emitted by the inflorescences or the green pods induce a partial termination of the reproductive diapause as has been observed

previously (Hodek, 1974; Hoxie & Wellso, 1983).

Table 1. Influence of inflorescences and pods on diapause termination.

Duration of the experiment	Frequencies of active insects after			
	10 days		20 days	
. Inflorescences	39 ♂ : 1	44 ♀ : 0,91	35 ♂ : 1	34 ♀ : 0,91
. Green young pods	55 ♂ : 0,83	57 ♀ : 0,67	32 ♂ : 0,85	33 ♀ : 0,82
. Mature pods	27 ♂ : 0,02	28 ♀ : 0	37 ♂ : 0	37 ♀ : 0
. Cowpea leaves	22 ♂ : 0	24 ♀ : 0	32 ♂ : 0	35 ♀ : 0
. Absence of vegetal organ	26 ♂ : 0	28 ♀ : 0	35 ♂ : 0	22 ♀ : 0

90-day old females in diapause were placed in contact with **V. unguiculata** inflorescences for 10 days. These females were then placed in the presence of sexually active males and dry pods under conditions of 12 h L 35°T. / 12 h D 25°C. for 15 days. Their reproductive activity (number of eggs, ovarian production) was compared with sexually active bruchids which had been placed under identical conditions immediately following emergence.

After 15 days the fecundity and the ovarian production of the post diapausing females is lower than in the control, but their reproductive activity is important. 71% of females produced more than 40 mature oocytes under experimental conditions.

The males that were in contact with **V. unguiculata** inflorescences for 10 days mated as soon as they came into contact with sexually active females; they emitted a spermatophore containing spermatozoa.

These experiments demonstrate therefore that **V. unguiculata** inflorescences indeed induce the reproductive activity of **B. atrolineatus**.

Table 2. Comparison of the reproductive activity of post-diapausing females and active females. The values of fecundities (t = 3.2) and ovarian productions (t = 4.4) are significantly different.

	Fecundities	Ovarian productions
Active females x active males (n = 41)	64.1 ± 5.5	74.1 ± 5.2
Postdiapausing females x active males (n = 38)	47.4 ± 8.2	51.9 ± 8.2

Analyses of inflorescence factors breaking diapause termination (Table 3). Contact with **V. unguiculata** pollen. Previous works (Pajni & Sood, 1975; Pescho & Van Houten, 1982) have demonstrated the role of the host plant pollen in other Bruchidae, for this experiment, male and female insects in diapause were placed in the presence of **V. unguiculata** pollen, renewed daily and provided with fresh flowers. This pollen is consumed by the insects (it has been found in the digestive tract) but there is no evidence of influence on diapause termination. Moreover, field observations show that **B. atrolineatus** adults do not enter the **V. unguiculata** flowers and do

not consume the host plant pollen in quantity.

Contact with extrafloral nectaries. Highly developed extrafloral nectaries are found at the base of **V. unguiculata** flowers on the floral scars (Ojehomon, 1968). The bruchids are highly attracted by these nectaries and consume the nectar which is rich in sugar (sucrose, glucose, fructose) and in amino acids (Baker & Baker, 1983).

Diapausing males and females were thus placed, for 10 and 20 days, in the presence of an inflorescence with all flowers removed. Only the floral scar and the nectaries remained. 70 to 75% of the bruchids became sexually active under these conditions. Contact with the nectariferous zone seems to provide induction of reproduction that could be due either to a trophic effect (the supply of sugars and amino acids causing an energy increase allowing the development of the reproductive organs), or to the action of allelochemicals acting as a signal.

Diapausing insects were placed in the presence of sugar solutions (each containing 15 g/l of sucrose, glucose and fructose) and amino acids (2 g each of alanine, arginine, serine, glycine). The insects consumed this sweet liquid but there was no evidence of diapause termination. Although this experiment did not reproduce the exact composition of nectar it demonstrated that the nectar influence is probably not linked to a trophic effect.

Contact with the corolla of the flower or the terminal part of the green pod. The diapausing bruchids in their boxes had access only to the corolla of the flower or to the terminal part of the pod. The calice and the extrafloral nectaries were not accessible. Under these conditions, development of the reproductive organs was observed, very rapidly when the insects were in the presence of the corolla, slightly more slowly when they had access only to the pod. It thus seems that the possibly active substances are not limited to the region of the nectaries.

Influence of the odour of the pods or of the inflorescences. The experimental boxes were separated into two compartments in order to prevent contact between the diapausing insects and the vegetal organs. Under these conditions, there was development of the reproductive organs during the experimental period. Odours of inflorescences have no influence on diapause termination.

Table 3. Influence of different inflorescence or pod factors on diapause termination.

	Frequencies of active insects after			
Duration of the experiment	10 days		20 days	
Inflorescences	22 ♂ : 0.85	24 ♀ : 0.97	20 ♂ : 0.95	24 ♀ : 0.92
Extrafloral nectaries	27 ♂ : 0.66	32 ♀ : 0.75	28 ♂ : 0.75	33 ♀ : 0.76
Corolla of flowers	36 ♂ : 1	38 ♀ : 0.87	34 ♂ : 1	32 ♀ : 0.95
Terminal part of green pods	32 ♂ : 0.65	36 ♀ : 0.62	32 ♂ : 0.68	26 ♀ : 0.70
Odor of inflorescence	40 ♂ : 0	44 ♀ : 0	45 ♂ : 0	42 ♀ : 0
Pollen of V. unguiculata	30 ♂ : 0	45 ♀ : 0	25 ♂ : 0	32 ♀ : 0

Discussion

These studies clearly demonstrate the role of the host plant reproductive organs in inducing sexual activity in **B. atrolineatus.** As with **Schistocerca gregaria** (Carlisle et al., 1965), the allelochemicals found on the vegetal surface (particularly the pods and flowers) probably induce diapause termination. These compounds are not present in the leaves and disappear with the maturing of the pods. The nature of these substances, the location of insect taste receptors and the mechanisms inducing reproductive activity have not yet been found. As with **Acanthoscelides obtectus** (Pouzat, 1981), it is probable that the gustative stimuli produced by the host plant are perceived by taste receptors located in the palpus. As opposed to other Bruchidae having imaginal diapause, **B. atrolineatus** adults hardly use the host plant pollen which does not represent a signal for the development of the reproductive organs. Rather, the adults consume the pollen which is more easily obtained from other plants in the biocenosis and the nectar form **V. unguiculata** flowers. This food allows the creation of energy reserves which will later be used for reproduction when diapause has been terminated. In the field, post-diapausing insects have abundant fat tissue and high fertility (Monge & Germain, unpublished).

Contrary to what has been observed in other tropical insects (Denlinger, 1986), the variations in the periodic factors (photoperiod, thermoperiod, hygroperiod) do not directly effect diapause termination (Germain et al., 1985). They can, however, modify the insect reponses to stimulating factors emitted by the **V. unguiculata** inflorescences (Germain, unpublished).

This regulation of the **B. atrolineatus** biological cycle by **V. unguiculata** inflorescences sets up a very precise synchonization between the reproductive cycles in the insect and in its host plant. Under these conditions, the insects are sexually mature when the seed pods begin to form. This regulation is very important in a region where the oviposition substrate (i.e., the pods of the host plant) is available for only a short time of the year.

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BRUCHUS AFFINIS AND THE FLOWERS OF LATHYRUS LATIFOLIUS: AN EXAMPLE OF THE COMPLEXITY OF RELATIONS BETWEEN PLANTS AND PHYTOPHAGOUS INSECTS

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Since Ehrlich & Raven (1964) introduced the concept of coevolution, phytophagous insects have been used to demonstrate mechanisms of coadaptation between plants and insects. G. Fraenkel (1959) had already stated that the "raison d'être of secondary plant substances" is an adaptation of plants to the attacks of insects. In most of the studies on this matter, attention is paid to the consumption of the plant tissues by insect larvae. Relationships between the adults of **Bruchus affinis** Frölich (Col. Bruchidae) and **Lathyrus** spp. (Leguminosae) prove that the mechanisms involved can be much more complex.

Lathyrus latifolius is a perennial semi-herbaceous vine which propagates by stolons. Pollination is by insects and the breeding system is essentially allogamous. Flowering and production of pods last three months (July, August and September) in the northern part of Béarn, France.

Bruchus affinis is a univoltin species. Adults emerge from pupae at the end of summer and live about 11 months, but they do not reproduce until they have undergone a reproductive diapause of nine months. Sexual activity and vitellogenesis do not start until the end of spring. At the beginning of summer, the females stick several isolated eggs on the young, green smooth pods of **L. latifolius, L. sylvestris** and **L. tuberosus.**

On hatching, larvae bore into the pods and then into the young seeds. As soon as the cotyledons have started growing, only one larva completes its development in each seed, after the elimination of the others. At the end of summer, after the dehiscence of the mature pods, a maximum of one adult emerges from each seed (Fig. 1).

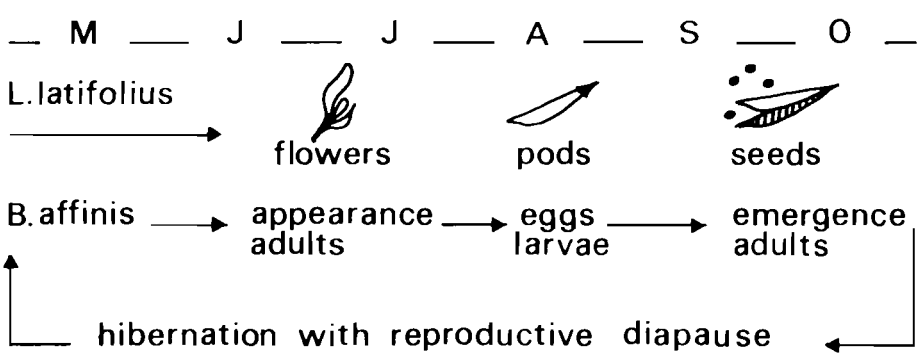

Fig. 1. Life cycle of **B. affinis** in relation to **L. latifolius.**

They show immediate activity; their fat body is whitish and very large while gonads are poorly developed. Although there are many adults emerging from the seeds, it becomes rapidly impossible to observe them on the late flowers of **L. latifolius** or on other flowers of the same colour. Adults emerging from seeds, kept in the laboratory, and confined with flowers of **L. latifolius** are not attracted to them.

A few adults were collected in winter under the bark of trees, near the populations of **Lathyrus**. Nevertheless, places where the adults gather during winter might exist. The bruchid numbers seem to be little reduced during the long period of reproductive diapause.

At the end of May, in Béarn, adults can be easily observed, for they visit the yellow flowers of **L. pratensis** in the neighbourhood of populations of **L. latifolius**. Their mouth parts are often covered with pollen. We have never found them feeding on the flowers of other plants.

Most of the adults collected in June on the flowers of **L. latifolius** have a large fat body. On the other hand, adults kept in cages outdoors, and fed with water and honey, have used all their reserves. Others, confined in petri dishes at 4°C ($\pm$ 1°C) still have a fat body similar to the one at the end of their larval development. Transferred to the outside temperature, they consume it rapidly.

Females of the adult stock and kept at 4°C, have been fed for more than one month with pollen of **Vicia sativa** and **L. pratensis**. Among the females fed with the pollen of **L. pratensis**, 15 of 22 showed pollen grains in their digestive tube and some of them (9/22) a whitish and abundant fat body. In the second experiment, the females fed with **V. sativa** showed no pollen grains and no fat body. Although they were confined with males, none of them were inseminated whatever the experiment (Bashar et al., 1985).

As soon as the flowers of **L. latifolius** appear, **B. affinis** adults leave the flowers of **L. pratensis** to reach those of **L. latifolius** (Fig. 2). One week after this move, some of the females collected in the field (6/15) contain mature ovocytes, a yellowish and abundant fat body, and they are inseminated. In the laboratory as well, females confined with males on **L. latifolius** flowers are inseminated, have an abundant yellowish fat body and ovocytes in retention (9 to 23 depending on the individual) (Table 1).

Expt.	number of B.affinis 0 / +	duration in days -	pollen in digestive tube -	fat body -	sperm in 0 / +	ovocyte per female retention -	ovocyte per female vitello -
labo	10	14	+++	yellow	+++	14,6 $\pm$3.8	18.1 $\pm$4.2
field	15	9	+++	yellow	+++	4.3 $\pm$2	10 $\pm$3.2

During the day, adults fly over populations of **Lathyrus** and direct themselves towards the flowers, on which they land frequently. On the other hand, they land indifferently on any vegetative structure of any plant in the site. They walk about on the mass of vegetation and seem to find the green pods of **Lathyrus** at random. Once a suitable pod is located the female explores it carefully before sticking on her eggs.

Therefore, the flower is the only structure capable of attracting bruchids to the plant and is attractive even before anthesis, and through the color changes during the flowering period. The nature of this attraction in unknown; chromatographic methods have revealed no terpenes.

B. affinis adults get into the flowers, while they are still green and closed, by opening the standard. In well opened flowers, adults can be observed deeply settled into the corolla and others opening the anthers.

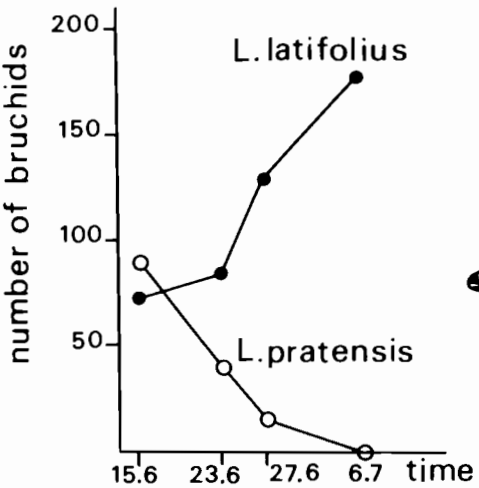

Figure 2. Displacement of **B. affinis** adults from **L. pratensis** to **L. latifolius.**

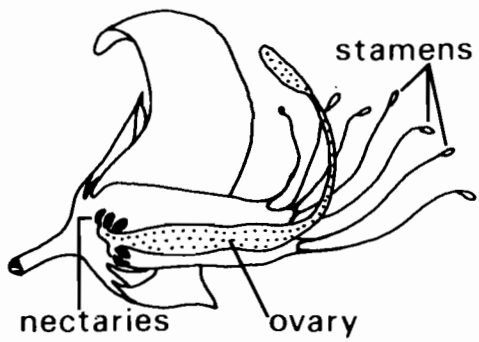

Figure 3. Cross section of a young mature flower of **L. latifolius.**

The flowers of **Lathyrus** have a ring of nectaries at the base of the staminal column, around the ovary (Fig. 3). Nectar has been collected, five times a day, at different dates, from flowers at different stages of development (unfortunately the damage done to the flowers does not allow the use of the same flower for several nectar collections). Nectar production starts before the opening of the flowers and soon becomes copious. Rate of nectar secretion and sugar concentration, both vary significantly with time of day and among inflorescences and plants (Hossaert et al., 1985).

The flowers are visited by **B. affinis** with a high frequency. In a **Lathyrus** population with a low density of **B. affinis**, two observers have followed, during 12h of a sunny day, (12.07.85, from 06h00 to 19h30 GMT with a break between 11h45 and 13h00, 44° lat.north-0° long day time from 04h34 to 19h40) the movements of the adults on 20 inflorescences (Table II).

For the duration of 10h15 of observation time, we recorded about 5 visits of **B. affinis** per inflorescence. The first visits occurred about 08h00. After 21h30, landings on flowers are not followed by new take-offs.

Just before nightfall, adults get into the flowers and remain there over night, one or several per flower; no movement of bruchids is recorded

Table II.

observation period	duration	No.landings observed	No B.affinis /h
8 am - 11.45am 1pm - 3.45pm	5 h 45	41	7.1
3pm - 7.30pm	4 h 30	65	14.4
total	10 h 15	106	10.3

during the night. When the weather is clear, flowers open within one hour after sunrise and, less than one hour later, **B. affinis** adults take off from the keel of the flowers. When the weather is cloudy, very few take-offs are recorded (Table III).

Table III. Observations on 20 inflorescences, 12.7.1985.

Night flights	take-offs before 08h00	diurnal visits	evening landings.
0	5	106	4

The number of adults reaching the flowers in the evening is similar to the number of adults escaping from them in the morning. This number is much lower than the number of the visits to flowers during the day. Two hypotheses can be proposed to explain these data:
a) if the adults visiting the flowers during the day and coming back to the same flowers to spend the night are the same; then the five residents observed previously would each make, on average, 20 visits during that time. According to this hypothesis, the same inflorescences would be used equally for resting and for feeding.
b) diurnal visits and nocturnal rests are independent with respect to the flowers. Some flowers would be used for resting and others for feeding.

The first hypothesis seems to be more likely, since independently conducted observations of adult behaviour, show that flowers are visited twice per hour.

This foraging activity of **B. affinis** adults on flowers of **L. latifolius** might have important consequences on the transportation and germination of the pollen, and on the fertilization of ovules as well.

Anthesis takes place two to three days before the stigma is receptive. After the opening of the anthers, the pollen grains fall down into the keel where they accumulate, but they remain able to germinate for five days. Therefore, the autopollen can be transferred to the stigma by insects moving about within the flower. Germination is then possible provided the surface of the stigma has been disturbed. The larger and more frequent are the insects, the greater the ease of penetration of the pollen into the style. Experimental manipulations have shown that repetitive intervention make the penetration easier (Valero et al., 1986). This seems to be necessary when there is no allopollen.

B. affinis not only can contribute to the transport of pollen to the flowers of this allogamous plant, it can also facilitate autogamy by moving

into the flowers when searching for pollen or nectar or sleeping inside the flower.

When visiting the flowers of L. **latifolius**, the adults of **B. affinis** can have effects on pollination at three different levels: stigmatic receptivity; transfer of autopollen from the bottom of the keel to the stigma; and bringing of allopollen. This "bringing" may not be uniform: the longer the bruchid stays in one flower, the higher the probability of its carrying pollen grains out of this flower: and we do not know if the same adults come back to the same flowers several successive nights and if the nectar of certain flowers is preferred to that of others.

One cannot forget that thrips stay in flowers as well and that numerous hymenoptera visit them. During the field observation, mentioned above, 137 visits of hymenoptera were recorded againts 106 of the bruchid. But what is worth considering, is the narrow specialization of the trophic relationship between the adults of **B. affinis** and the flowers of L. **latifolius**. Only the flowers of three species of **Lathyrus** (**L. latifolius, L. sylvestris, L. tuberosus**) are capable of stimulating the gametogenesis of **B. affinis**. This specialization can be a contribution to the reproduction of these species.

At a first glance, it seems that **B. affinis** profits to the **Lathyrus** and conversely that, of course, **Lathyrus** entertains **B. affinis**.

Before concluding that a new example of coevolution exists between plants and insects, one must not forget that bruchid larvae are the only insects able to feed on the growing seeds and that in certain locations, 90% of them can be destroyed in such a way. Consequently it seems necessary to put the question: what is the importance of increasing the pollination efficiency in a plant species that reproduces also by stolons?

Before drawing any conclusion on the adaptative value of the attractiveness of the flowers for the bruchids, it is necessary to stress the importance of the relationships between the two species, and by viewing this in context to the ecological requirements of these two elements of the biotic community.

Finally, if one wishes to avoid seeing an adaptative advantage in every trait of the behaviour of an insect, it is necessary to recognise, as did Jacob (1981), that no adaptation can be perfect because it is a result of "tinkering" imposed by historical constraints. This "panglossism", using the term of Gould & Lewontin (1979), can only obscure our jugment and bias any attempt of analysis.

Acknowledgements

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DIFFERENT FOOD SUPPLY STRATEGIES IN MIDGE INDUCED PLANT GALLS

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1. Introduction

Plant galls can always be distinguished from other growth and developmental anomalies by the fact that a specialized nutritive relationship exists between the gall inducer and its host plant (Küster, 1911). This relationship is usually demonstrated by the presence of a nutritive tissue or of nutritive cells which indicates an active reaction of the host plant. Phytophagous gall midge is considered to be a modern phytophagous taxa which was triggered by explosive evolution of angiosperm taxa (Zwölfer, 1984; Roskam, 1986). Gall midges constitute the most important group of gall insects (Felt, 1940), and they have a very large host plant range. Their galls range from simple leaf folds to ingeniously constructed structures. Phytophagy and gall inducing habit are believed to have evolved from primitive mycetophagy (Mamaev, 1975). Among the gall inducers some groups (all **Asphondiliini**, many **Lasiopterini**) are phytomycetophagous; the larvae feed on fungi which they breed in the larval cavity (Bronner, 1977). The modern representatives of the tribes **Oligotrophini** and **Cecidomyini** lost the link with fungi, they are phytophagous, specialized plant eating parasites. Their oral apparatus is adapted to piercing and sucking plant cells.

In this paper the purpose will be to present two different gall types of the tribe **Oligotrophini**; two different feeding strategies are used by the gall insect to derive soluble food from the host plant. One type, the most usual among the phytophagous gall inducers is represented by **Geocrypta galii** Lw. on **Galium mollugo** L.. The other type, a more original one is represented by **Didymomyia reaumuriana** Lw. on **Tilia** (**T. cordata** Mill. x **T. platyphyllos** Scop.). Both galls are covering galls; the first instar larva begins its activity on the host epidermis cells; it becomes covered by tissues which grow around it.

2. Materials and methods

The galls of **Didymomyia reaumuriana** Lw. are collected on a lime tree near Strasbourg. The galls of **Geocrypta galii** Lw. are reared in the laboratory on the host plant (Rohfritsch, 1971).

The classical method of fixation and embedding of the material for electron microscope observations of ultrathin sections are used (Rohfritsch, 1971). To have a better representation of the three-dimensional aspect of the different cell organites, thick sections are observed after selective impregnation of the material with zinc iodide and

osmium tetroxyde (see the technic in Marty, 1973; Rohfritsch, 1976).

3. Results and discussion

1. Gall initiation

Upon hatching the gall midge larva locates the right place and begins to "attack" the epidermis cells of the host plant. A day later drastic cytological changes can be observed in these cells and in one or two cell layers beneath: there is homogeneization of the tissues. The cuticula is no longer synthesized, and the cell wall remains thin. The area of attacked cells present no cell rupture, no cell death. Instead the cells are activated to proteosynthesis and to RNA synthesis. The nucleus is large and is located in the center of the cell. Its nucleolus is also large and the chromatin appears extended. Small flexuous plastids, free of starch, form a crown around the nucleus. The cytoplasm is dense, rich in ribosomes, dictyosomes and rough endoplasmic reticulum. Numerous small vacuoles appear. These modifications of plant cells have ben called "metaplasia" by Meyer (1957).

2. Gall development and tissue differentiation

All around the place where the larva is active, cells begin to divide, building a wall around the insect. Trichomes appear on the epidermis covering the wall. The young larva controls the enveloping growth (Rohfritsch, 1971).

a) As it is usually the case on herbs, the gall development of **Geocrypta galii** is fast: after 3 days, the larva is totally enclosed in the larval cavity. Cell growth is mainly inhibited between the vascular bundles of the attacked stem and the larval cavity. The cavity is large and communicates with outside air through the ostiolar channel. The gall, the larval cavity and the larva develop regularly at the same rate, in 2 weeks. The larva is mobile, and moves continuously along the large surface of the nutritive tissue cells. At the bottom of the cavity, only five cell layers separate the larva from the vascular bundles of the young stem (Fig. 1a).

b) **Didymomyia reaumuriana** needs 5-6 days to be covered by the growing tissues, and 5 weeks to reach the adult size (Rohfritsch, 1971). The active tissue growth isolates the larval cavity from the vascular bundles of the host plant. In the nearly full grown gall the larva remains as small as in the early stages; it is deeply bedded in a mass of white parenchyma. The ostiolar channel becomes very thin. The tissues around the larva are parenchymatous as long as the larva does not feed much. When the larva needs a high quantity of food (2 weeks before maturation) a nutritive pad differentiates; distally around it a sclerenchyma layer is formed (Fig. 2a).

3. The two types of nutritive tissue.

a) The nutritive tissue of **Geocrypta galii**, composed of 5-8 cell layers is in direct contact with the vascular bundles of the gall or on the bottom of the larval cavity with the phloem of the attacked stem (Fig. 1a) where the nearly mature larva feeds preferentially. The nutritive tissue covers the inner wall of the larval cavity excepted the ostiolar area.

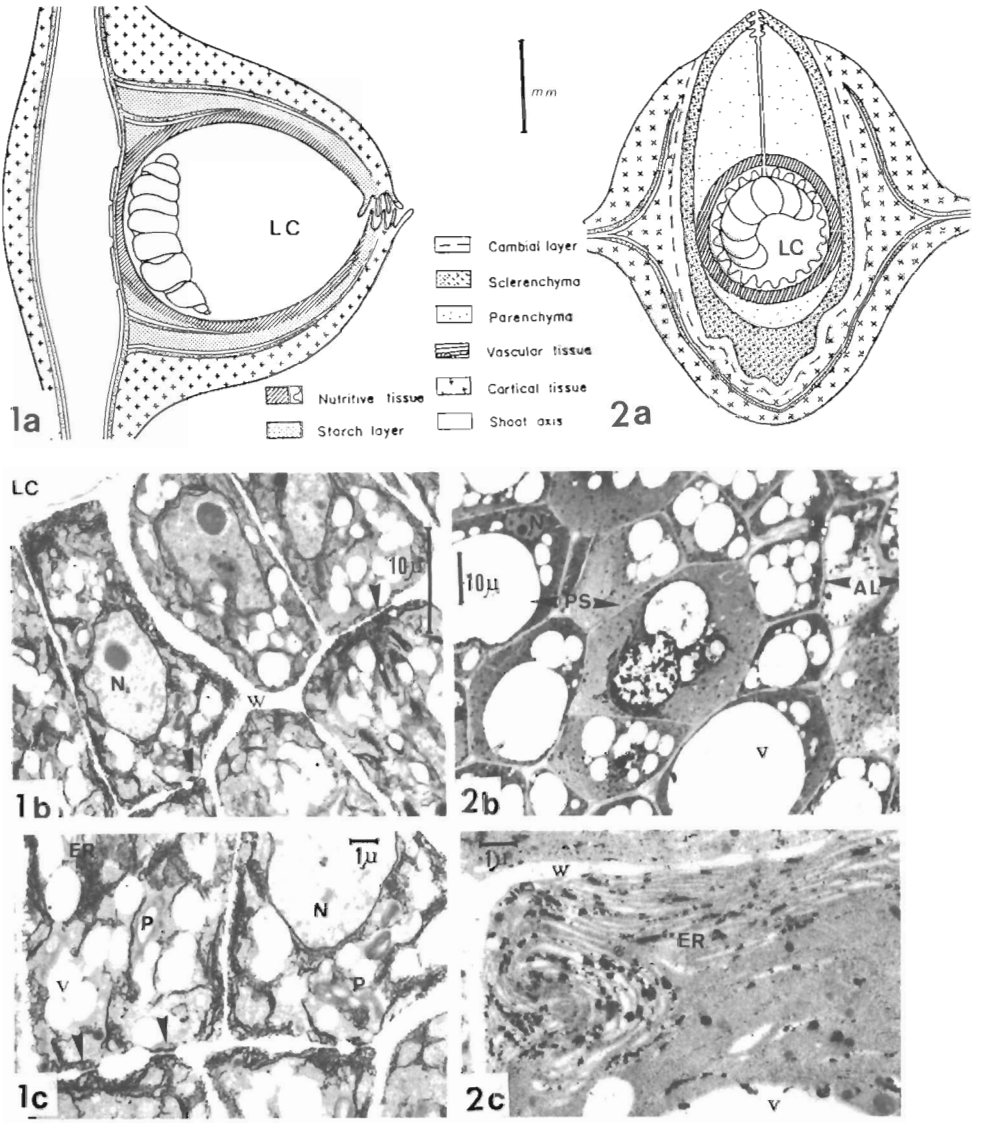

Figure 1. The gall of **Geocrypta galii** Lw. on the stem of **Galium mollugo** L. 1a. Schematic diagram of a mature gall. 1b. Cells of the first and second layer of the nutritive tissue of a half grown gall. 1c. Detail of Fig. 1b. A very abundant endoplasmic reticulum (ER) essentially located along the cell wall communicates from cell to cell through numerous plasmodesmata, gathered in large pit fields (arrow) W: cell wall.

Figure 2. The gall of **Didymomyia reaumuriana** Lw. on the leaf of **Tilia** (**T. cordata** M. x **T. platyphyllos** Sc.). 2a Schematic diagram of a mature gall. 2b Nutritive tissue cells in various stages of cell activation to proteosynthesis (PS) or to autolysis (AL). 2c. Detail of the cytoplasm of the activated cells: the endoplasmic reticulum forms large stacked cisternae with a dark content, the membranes are not contrasted by the mixture of zinc iodide and osmium tetroxyde. N: nucleus; LC: larval cavity; V: vacuoles; P: plastids.

The major cytological characteristics of these nutritive tissue cells include the presence of an abundant endoplasmic reticulum, essentially, located along the irregularly thick cell walls (Fig. 1b, c). Mitochondria constitute the second dominant element. The cells present a relatively large nucleus with an important nucleolus; excretory types of vesicles are present in contact with the plasma membrane. The different cell layers between the larval cavity and the vascular bundles commnicate through the endoplasmic reticulum via numerous plasmodesmata preferentially oriented and gathered in large pit fields. A very important symplastic transport occurs between the vascular tissue of the gall and the larval cavity.

The same kind of transport along a short distance pathway is described in nectaries of **Abutilon** (Gunning, 1976). The transport of sugars and phloem unloading occur through a high number of plasmodesmata and a dense endoplasmic reticulum. An "explosion" process is described in which ER cisternae distend and liberate their contents, through their ownlimiting membrane and the plasma membrane. It has not been observed that drops are spontaneously secreted by the nutritive tissue cells of **Geocrypta galii**. However, such a "secretion" could be produced under the influence of the sucking larva.

b) The nutritive tissue of **Didymomyia reaumuriana**, composed of a great number of cell layers, is formed by active cell proliferation; it constitutes a mass of tissue around the larva which isolates the larval cavity from the vascular tissue of the attacked organ (Fig. 2a). This mass of nutritive tissue develops in the early stages of cecidogenesis, at the time the young larva and the larval cavity remain very small. It will be consumed by the maturing larva. The larva will draw on the solutes, the reserves and even the protoplasm of the cells for nutrients.

The cell contents become hydrolyzed from the centrally located nutritive tissue (in contact with the larva) progressively toward the hard layer. Before becoming lyzed the cells are stimulated to proteosynthesis (Fig. 2b). They are believed to prepare the necessary enzymes for cell autolysis.

As the larva matures, the layers of activated cells progress toward the periphery of the nutritive tissue. The solutes, to reach the larval cavity, have to cross more and more layers of depleted cells, they migrate essentially in an apoplastic way.

This type of nutritive tissue is composed of cells in different stages of autolysis and cells activated to proteosynthesis. The major cytological characteristics of this last cell type is an abundant endoplasmic reticulum forming large stacked cisternae. Often the cisternae are swollen, they have only little contact with the cell wall or with the nucleus. The selective impregnation with zinc iodide and osmium allows to show a dense content in the cisternae. This content appears to be partially of Golgi origin (Fig. 2c).

The nucleus and nucleolus are small, and the very small plastids are gathered in one or two packets in the cell. Mitochondria are poor, long, filamentous, and essentially located along the cell wall.

The cell wall remains thin, regular, and the plasmodesmata are randomly distributed. Cell wall lysis is not very important during the

process of cell autolysis, so that the empty cell walls accumulate aroud the larval cavity (Rohfritsch, 1986).

This type of nutritive tissue should not be regarded as a unique phenomenon since the physiological and cytological characteristics are similar to endosperm cells during the embryonic development, as described on wheat (Smart & O'Brien, 1983).

In both cases, there is a first stage of active growth and tissue development, reserves are accumulated, the tissue functions as an attractor. In a second stage, the reserves and even the protoplasm of these cells are hydrolyzed in favour of the larva or of the embryo. The cell walls which are not destroyed constitute layers of depleted cells, the solutes migrate essentially via the apoplast.

4. Conclusion

These two main types of nutritive tissues considered from a functional stand point, the first appears as a short distance transport tissue, through the symplastic way between the vascular tissue (essentially the phloem) and the larval cavity. This nutritive tissue can be more or less extended around the larval cavity; it appears always as grafted on the vascular tissue of the attacked organ. The insect has access to the vascular tissue of the host plant. It represents the main type among Cecidomyidae. The second type of nutritive tissue acts first as an attractor, i.e. an active converter of simple molecules into immobile molecules, and later as a food provider, by hydrolyzing its reserves and its cytoplasm. The apoplastic way seems to be the most important for solutes transport, the cells become leaky before they are lyzed. In this second case a very important cell proliferation isolates the insect from the vascular tissue of the host organ. The formation of a sclerenchyma or hard layer achieves this isolating process.

Abstract

The host plants of gall-midges are provoked into surrounding feeding sites with specialized cells which provide the insect with nutrition. Two main types of nutritive tissues are presented. The first type, the most common, is composed of only a few cell layers which separate the larva from the vascular tissue of the host. The cells on which the larva feeds are never destroyed, their structure and cytological features point to a specialization for a rapid, short distance solute transport. The second type, composed of numerous cell layers, constitutes a nutritive pad around the larva. The cells of the pad will be consumed by the last instar larva. The larva will draw on solutes, on reserves and even on the protoplasm of the cells.

Key-words: Galls, Cecidomyiidae, Oligotrophini, nutritive tissue.

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GROWTH REGULATION OF PHRAGMITES AUSTRALIS BY THE GALLMIDGE GIRAUDIELLA INCLUSA

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1. Introduction

The induction of galls is a highly specialized form of phytophagy with characteristic features. Above all, the stationary way of life causes other consequences than the habit of free-living phytophagous insects.

During my studies in the population ecology of **Giraudiella inclusa** I focused on the interaction between this gallmidge and its hostplant common reed (**Phragmites australis**). With respect to the hostplant growth regulation the following questions arose:
(1) How do the gallmidge-larvae provide themselves with sufficient nutrients - in spite of the immobility during their food intake?
(2) Do the modifications at the place of infestation have influences on the reed stem growth?
(3) Are the consequences of this interaction favourable or detrimental for both the gallmidge and the host plant?
(4) What is the relative importance of this insect-plant relationship within the food web interactions of the **Giraudiella**-populations in the reed-ecosystem?

2. Methods

Along the Elbe near Hamburg different stands of **Phragmites australis** were investigated from 1981 to 1984. As far as possible all important interactions between the gallmidge **G. inclusa** and the other organisms of the reed-ecosystem were analyzed. Quantitative studies of several reed parameters pointed to the reasons of the **Giraudiella** infestation pattern; chemical analyses and experiments gave further evidence (for details see Tscharntke, 1986).

3. Results and discussion

3.1. The biology of **Giraudiella inclusa**

G. inclusa forms characteristic clusters of grain-like galls inside the reed-internodes (Skuhrava & Skuhravy, 1981). There seemed to be 4 generations, each of it separated in fast developing individuals and individuals that remain as larvae 3 until hibernation. The whole population pupated and emerged during May of the following year synchronously. The first generation of **G. inclusa** attacked the 1st to 9th internode, the 2nd to 4th generations infested stepwise the 10th to at most the 23rd internode, which are the newly grown internodes during the summer. The holes of the emerging gallmidges and parasitoids were the only external

signs of the **Giraudiella**-attack on the reed stem.

The females deposed clusters of eggs on the latest developed internode on the top of the reed stem, strictly speaking they were layed on the ligula, localized on the transition between the leaf sheath and the leaf blade. The egg larvae migrated between the leaf sheath and the internode-surface of the youngest internode in basal direction, where they started nidification with the help of gall-inducing secretions. Experiments showed that the young larvae orientated themselves by the chemical differences along the internode. Nidification only took place in the basal area of the internode, the region with the strongest cellular growth, with the highest content of nitrogen, sugar, minerals and water, and with the lowest content of detrimental substances, especially raw fiber and silicate.

The **Giraudiella**-females adapted the number of layed eggs to the basal shoot diameter. The clutch size of eggs significantly depended on the stem-thickness.

3.2. Interactions between the populations of 4 trophic levels

The investigation of the **Giraudiella**-populations as a part of the food web in the reed-ecosystem showed important interactions between the populations of four trophic levels.

The key factor for the abundance of the **Giraudiella**-populations was the resistance of its hosplant common reed. Wet habitats with thick and silicate-rich reed stems bore less galls than dry reed with its thin shoots. 82% of the gallnumber-variance from the first **Giraudiella**-generation were explained by the average stem thickness of the habitat. 92% of the deposited eggs in a wet stand didn't result in galls; the high loss was due to the egg larvae, because the hardness of the silicate-rich tissue obviously inhibited the nidification of the larvae.

Associated plants in the dry reed-polycultures seemed to be responsible for reduced egg-deposition on reed stems ("associational resistance"). The temporarily great damage caused by the stem-mining caterpillars of **Archanara geminipuncta** lead to reduced reed-resistance and a large increase of **Giraudiella**-galls in the wet habitats. During the winter the blue tit (**Parus caeruleus**) killed a high percentage of the gall inhabitants (about 60% of them were parasitoids).

These different types of (only the most important) interactions may illustrate, that it is necessary to analyse the complex relationships between four trophic levels, before assessing the poulation ecology of a phytophagous insect or before assessing the relative importance of each type of interaction (e.g., Price, 1985).

3.3. The stimulation of food increase and reed stem elongation

The effects of the **Giraudiella** attack on the reed stem growth were habitat-specific.

(1) In wet reed monocultures with their low infestation-level **G. inclusa** didn't cause modifications to the stem growth. The **Giraudiella**-larvae successfully causing galls didn't seem to influence the linear growth of the thick and silicate-rich stems.

(2) The occurrence of **Giraudiella**-mass attacks with heavy damage to the host plant was limited to a waterstressed reed-monoculture growing in brackish water.
(3) In the widespread dry reed polyculture the moderate infestation-level of **G. inclusa** had quantifiable consequences for the host plant growth. The length of those internodes that have not been infested by phytophagous insects was measured to get "standard internodes" of normal stems. These lengths of unattacked internodes were compared with **Giraudiella**-infested internodes of the same thickness and the same height (Fig. 1). On an average the infested internodes of the first **Giraudiella**-generation were 1.12 times longer and that of the 2nd to 4th generations were even 1.29 times lengthened. The internodes grown above the point of **Giraudiella**-attack were elongated too. Vice versa the situation of the 2nd to 4th generations: here the internodes below the infested internode were longer.

There is a fairly obvious explanation for this phenomenon: the gallmidge **G. inclusa** caused not only a local effect which is limited to gall formation as usual, but it induced a prolongation of the whole internode by means of a high level of plant hormones. The plant hormones, probably auxins and gibberelins, were transported by the assimilate flow. Therefore the internodes following the direction of the assimilate-flow got a stimulation of the plant growth regulaters as well. During the development of the 2nd to 4th generations the assimilate flow was already reversed in the direction of the reed-rhizomes. The internodes grown in the shade of the assimilate flow didn't reach their normal length.

The influence of **G. inclusa** on the reed growth was not limited to the internodes. Even the whole reed-stems were significantly elongated when infested with galls. The prolongation was 7% to 11% as compared with unattacked standard stems.

The attacked plant tissue showed a concentration of compounds which was obviously caused by the increase of plant hormones, because plants especially send their assimilates to places of the highest hormone-level. The effect was studied for internodes with 4.4 galls and a 7% prolongation on an average. The infested internodes had 23% more fresh weight, 32% more dry weight, and 36% more nitrogen than unattacked "standard internodes".

3.4. The significance of the growth regulation

One aspect of the prolongation is it's consequence for the infested reed stem. Obviously the elongated shoots got more light than their shorter neighbours. Light is a limiting factor for the rate of photosynthesis in **Phragmites australis** and beyond that the highest photosynthetic intensity takes place in the upper leaves. There is a strong intra- and interspecific competition for light within reed-stands (e.g., Van der Toorn & Mock, 1982). The prolongation and the increase of dry weight following the infestation of **G. includa** suggest that the gallmidge didn't damage its host plant in the dry reed polyculture but stimulated a higher productivity. The compensatory growth seemed to be orientated to a gain in competition for light. (However there must be reviewed a lot of unknown factors before judging the total consequences for the plant fitness, see Janzen, 1979).

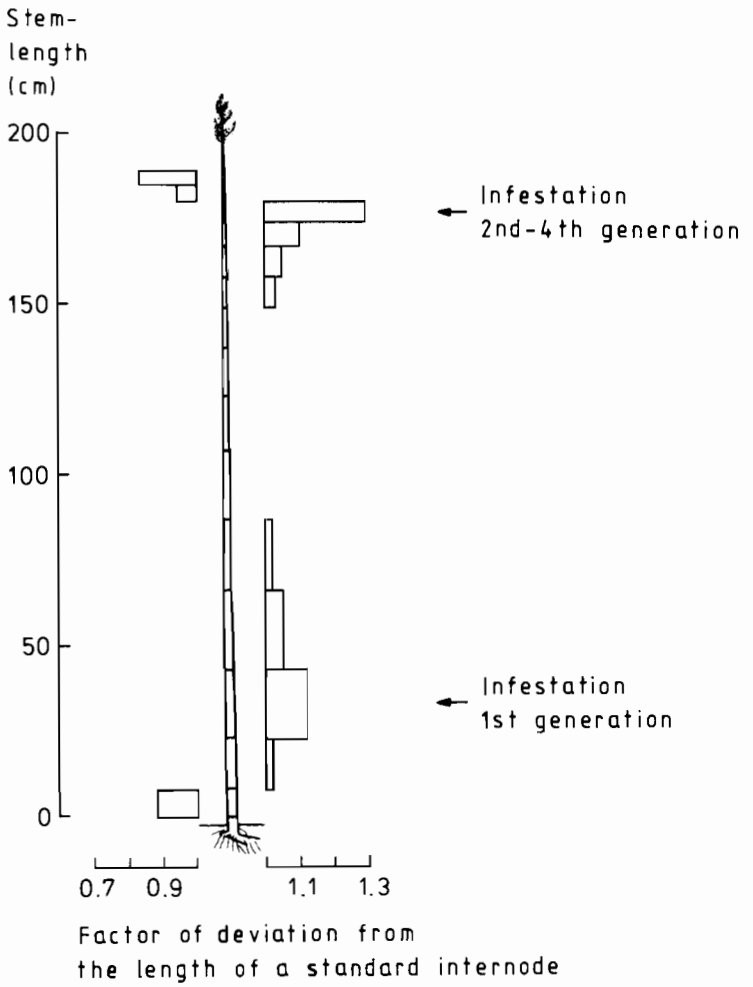

Figure 1. The influence of **G. inclusa** on the linear growth of **Phragmites australis**. The arrows indicate the mean value of the attacked internode-height (for details see Tscharntke, 1986).

The induction of the internode-prolongation should be advantageous for the gallmidge too. The numerous galls inside the elongated internodes apparently developed better because of the larger space. The increase of biomass and nutrients improved the food quality and quantity. Furthermore the **Giraudiella**-females adapted the number of eggs per oviposition to the basal shoot diameter, as shown above; thick stems got more eggs per cluster than thin stems. The growth regulation and the pattern of egg-laying obviously resulted in a sufficient food basis for the crowded consumers.

A negative correlation between larval weight and gall density couldn't be found. Accordingly the possible negative consequence of aggregate habits, namely the intraspecific competition, could obviously be devoided.

The material concentration by diverting the plant assimilates to the points of gall-formation (e.g., Bronner, 1977; Weis & Kapelinski, 1984) as well as a high precision in egg-laying (see Ahman, this volume) are characteristic features of gall insects in comparison with free living phytophagous species. The stationary living of the larvae requires that the females choose (1) a suitable hosplant, (2) an adequate time and place for egg deposition, (3) a favourable clutch size, and that (4) the host plant can be forced (mainly by the larvae) to make a local nutrient concentration available sufficient for all the consumers inside the gall. Due to their immobility galling larvae cannot search actively for places of high nutient levels; they depend on the oviposition decision of their mothers and the host plant response to their specific secretions.

The necessity of exact synchronisation with suitable host plant tissues and the subtle host growth manipulation represent a particularly intimate plant-insect relationship, so that gall-inducers should be highly vulnerable to plant defence mechanisms. Host plant-resistance may be the key factor in population dynamics not only of the gallmidge **G. inclusa** but of most other galling insect-species too.

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THE EFFECTS OF CEUTHORRHYNCHUS NAPI (CURCULIONIDAE, COLEOPTERA) ON STEM TISSUES OF BRASSICA NAPUS VAR OLEIFERA

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1. Introduction

The weevil **Ceuthorrhynchus napi** Gyll. provokes growth abnormalities on rape stems characterized by flattening, swelling of various sizes and longitudinal splits. In spite of many studies, the true causes of these injuries remain unknown. According to previous papers, the plant's reactions could be due to secretions from the female during oviposition (Gunthart, 1949; Deubert, 1952), to substances arising from the eggs (Dosse, 1955), to the swelling of the egg which attracts fluids from the surrounding plant tissues (Deubert, 1955) or to bacterial transmission by the female (Kazda, 1958).

We tried to define the influence of **C. napi** on the rape stem tissues in a large study from the oviposition stage until the dropping to the ground of the full grown larvae (*).

The purpose of this paper is to analyse modifications caused by **C. napi** on the rape stem tissues from the time of egg laying until the egg hatching which occurs within 6 days under our experimental conditions.

2. Materials and methods

Winter-rape (**Brassica napus** L. var **oleifera** Metz. cv "Jet-Neuf") used in culturing **C. napi** were collected in fields, and potted plants were placed in a growth chamber under a photoperiod of 10 h light and 14 h dark at a temperature of 20°C. Mature females after mating were caged with plants. Ovipositional behavior was observed and shoots with eggs at various stages of development were collected for cytological studies. Small pieces of tissues were treated using classical methods for electron-microscopy. For observations with an optical microscope, semi-thin sections were treated with toluidine blue.

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* This work is part of an unpublished thesis: defended by H. Le Pape: "Dommages agronomiques et histocytologiques provoqués par la ponte de **Ceuthorrhynchus napi** sur le colza - Caractérisation et recherche de leurs origines" (E.N.S.A.I.A., Nancy, France, 1986).

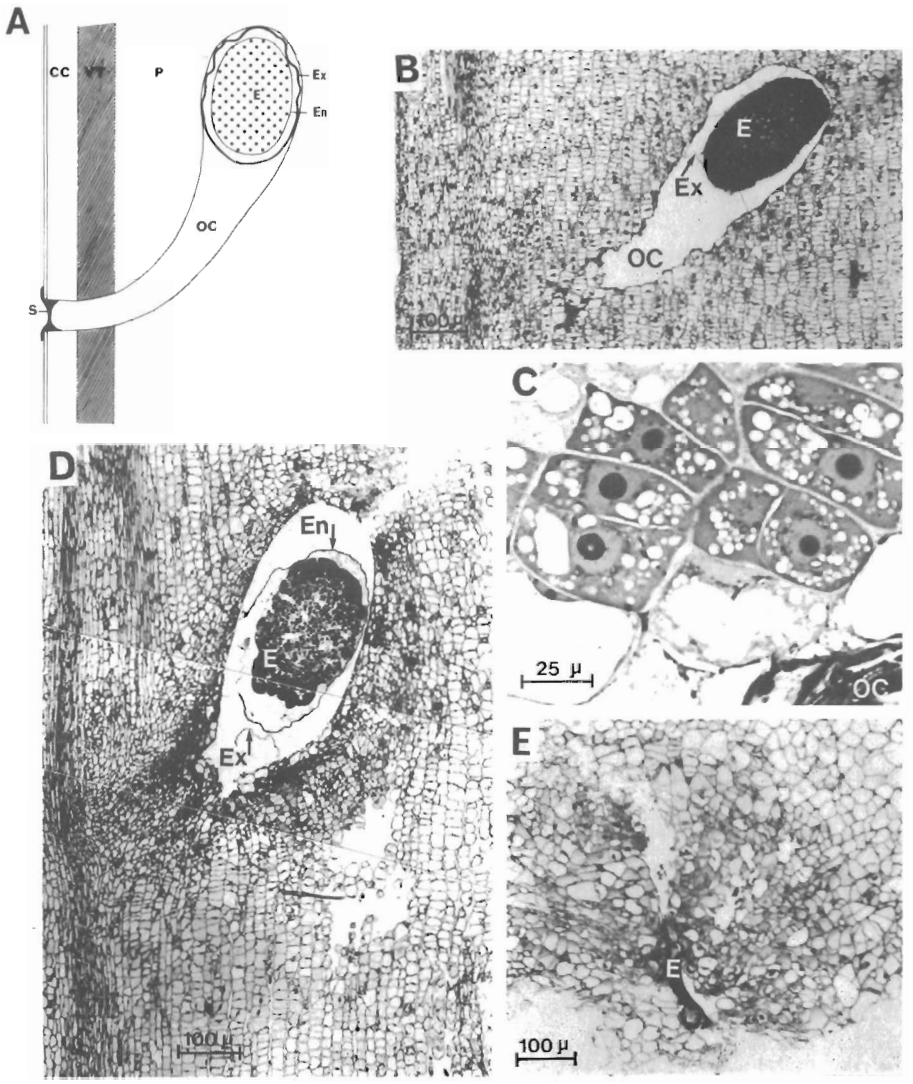

Figure A. Diagram of a longitudinal section of a rape stem showing the oviposition channel (OC), with its opening sealed by a substance (S), the egg (E) surrounded by the endochorion (En) and the exochorion (Ex); CC: cortical cells; P: pith; VT: vascular tissues. **Fig. B.** 1 day after oviposition. **Fig. C.** 2 days after oviposition: cell content modifications and cell divisions. **Fig. D.** 5 days after oviposition. At the top of the nodule surrounding the egg a break appears. The exochorion is fragmented. Many holes appear in the pith around the nodule. **Fig. E.** 6 days after oviposition, the egg leaves the nodule, cell growth tends to fill the egg chamber.

3. Results

3.1. Oviposition behavior - Observation of the oviposition channel

The female searching for oviposition site moves about on the plants, tapping the surface with the rostrum. When suitable site is found, the female positions with the head facing downwards. In this position it chewes a channel in the stem with the mouthparts situated at the tip of the rostrum, ingesting plant tissues at the same time. Then, it turns halfway around and with its ovipositor deposits a single egg into the channel.

The oviposition channel is obliquely oriented towards the tip of the stem and its top, situated inside the pith, forms an expanded chamber wherein the egg lies (Fig. A). Both the oviposition channel and the egg chamber are lined with crushed cells. The opening of the channel is sealed by a substance secreted by the female. Soon after oviposition, a membrane-like structure (presumably the exochorion according to terms used by Seifert, 1970) separates from the egg and goes to adhere to the plant cells. This membrane lines the wall of the egg chamber, forming a kind of closed pouch around the egg.

3.2. Histological observations on the stem tissues

- 2 hours after oviposition: in some of the young pith cells slight modifications of the cell content are detected: the nucleus enlarges, becomes centrally located, the perinuclear plastids accumulate starch.
- 12 hours after oviposition: the nuclear modifications observed in some cells become more intense and are accompanied by greater abundance of cytoplasm, nucleolar hypertrophy, and fragmentation of the large central vacuole into smaller vacuoles.
- 1 day after oviposition: some of the cells nearest the top of the egg chamber and the cells of the vascular tissues present most intense cell activation with hypertrophied nucleus and nucleolus, dense cytoplasm, many very small vacuoles (Fig. B). The first divisions occur.
- 2 days after oviposition: in tissues situated mainly up above the channel and the egg chamber, many cellular divisions without preferential direction are observed and so the pith cell arrangement is deeply modified. In the vascular tissue area earlier cut by the female, activated cells begin to proliferate towards the hole of the channel (Fig. C).
- 3 and 4 days after oviposition: the channel is now filled by proliferating cells of the vascular area. Around the egg chamber lined by the exochorion, a heterogeneous mass of cells occurs among which the number of activated cells increases. Cell division is pericline to the egg chamber.
- 5 and 6 days after oviposition: the channel is now completely obstructed, but the egg chamber remains large, and unobstructed by cell growth. The inner limit of cell proliferation coincides with the localization of the exochorion. In the cortical area, the channel remains open, and no cell proliferation is observed. The mass of more of less modified cells forms around the egg chamber a kind of heterogeneous nodule. This nodule is surrounded by the pith cells where cell maceration is beginning (Fig. D). The differential growth rates of stem tissues create many holes in the pith near the nodule. So, the nodule becomes weak, and it breaks at the top of

the egg chamber in an area of parenchymatous cells (Fig. D). At this stage, the exochorion is fragmented. The egg leaves the nodule, pushed into a hole of the pith by the cell growth which tends now to fill the egg chamber (Fig. E), and also by movements of the embryon inside the chorion. Upon hatching, the larva tunnels into the developing stem destroying the pith.

4. Discussion

The observations of the modifications caused in rape stem tissues by the oviposition of **C. napi** allow to emphasize:

- the part of the oviposition wound in the first plant reactions,
- the role played by the exochorion in the non-obstruction of the egg chamber,
- the action of the egg and the developing embryo.

4.1. Part of the oviposition wound in the first plant reactions

The first reaction of the plant after oviposition corresponds to a modification of the cell content without division. This modification corresponds to a dedifferentiation of the cellular structures and demonstrates stimulation of the cell metabolism. This phenomenon looks like metaplasia as it is observed in the first development stages of many insect galls (Meyer & Maresquelle, 1982). In most insect induced galls, metaplasia generally appears first in plant cells directly under the influence of the cecidozoa, and many contiguous cells are affected. In the case od **C. napi**, the metaplasia is heterogeneous: many normal cells are found between modified cells, both in areas nearest the egg and in areas farthest from the egg. This observation leads to the hypothesis that the modifications should be essentially due to the wound made by the female before oviposition. Indeed, these modifications are similar to cell dedifferentiation observed by Barckhausen (1978) in wounded storage tissues. But in the case of **C. napi**, the cell dedifferentiation is accompanied by a starch synthesis perceptible as early as 2 h, whereas in storage tissues the wound induces a starch regression (Barckhausen, 1978). On the other hand, the wound caused by the female of **C. napi** induces reactions in rape stem, comparable with those observed by Kupila-Ahvenniemi (1966) in the case of wounded bean stems. The hypothesis of a wound reaction in the case of **C. napi** is experimentally corroborated. Indeed, in rape stem wounded by a needle, or in rape stem where a female only chewes the channel and the egg chamber without inserting the egg, we observed a similar plant reaction: cellular activation and starch synthesis (Le Pape, thesis). According to Thorpe & Meier (1972) starch synthesis in cells near a wound corresponds to a flow of metabolites before cellular divisions in order to heal the wound.

So, as no substance secreted by the female of **C. napi** around the egg has been observed during the oviposition act, it is thought that the first plant reactions are caused by the oviposition wound. Afterwards the cellular modifications are maintained by egg activity, because without egg, cell proliferation is very rapid and cellular activation quickly regresses (Le Pape, Thesis).

4.2. Role of the exochorion

In the egg chamber lined by the exochorion, there is no cellular proliferation. When a female chewes a channel without inserting the egg, the egg chamber is soon filled by cellular proliferation within less than 4 days (Le Pape, thesis). At the end of the egg development, when the exochorion is broken, the egg chamber is obstructed. These observations suggest that the exochorion could act as a protection of the egg against plant cell proliferation. The exochorion could have a "anti-healing" action. The exochorion has been erroneously interpreted by Deubert (1952) and earlier workers as a substance secreted during oviposition. Experiments are needed to know more precisely the chemical nature of this membrane and to clarify its action in relation to the egg part. Preliminary histochemical tests show that the exochorion could be composed of glycoproteins. The exochorion seems to act as an interface between the plant cells and the egg, permitting the plant cells to be receptive to the egg action.

4.3. Action of the egg

Experimentation showed that the egg does not act by means of lytic enzymes (Le Pape, thesis). During embryogenesis the egg swells by attracting fluids from the surrounding plant tissue (Deubert, 1955). The presence of the egg creates a kind of sink for water and solutes and the egg is responsible for the formation of the nodule of small and metabolically active cells around it. There are modifications in the osmotic balance of the stem tissues which lead to differential cell growth in the pith tissue around the nodule. Both cell growth and movements of the embryo inside the egg shell participate in the formation of breaks which allow the egg to leave the nodule and to reach the pith channel. The lytic process which can be observed around the nodule is of the same kind as that which is observed in the pith of normal plants. Our observations emphasize the importance of the action of the egg in the phenomenon observed a stated by Deubert (1955). As mentioned before, there is no subtance secreted by the female during the oviposition. This contradicts results of Gunthart (1959) and Deubert (1952) who thought that substances presumably coming from the female could play a part. Further experiments are needed to understand better the influence of the egg and of the exochorion.

Acknowledgements

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Labeyrie, V., Fabres, G., Lachaise, D. (eds) Insects – Plants

ECOLOGICAL SIGNIFICANCE OF WOUND-INDUCED CHANGES IN PLANT CHEMISTRY

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1. Introduction

Physical injury to leaves causes a wide range of changes, both physical and metabolic, to the plant tissues. Some of these changes, for example increased water loss or local changes in nutrient cncentrations, may be inevitable consequences of the disruption of tissues. Others, such as alterations in levels of plant hormones, increased respiration and protein synthesis, are clearly part of the repair process. However there may also be changes in levels of secondary compounds which have no obvious metabolic significance, and these effects often also appear in tissues remote from the site of damage ((Edwards & Wratten, 1983, 1985).

The idea that secondary metabolites whose synthesis is promoted by damage may represent an active defence against insect herbivores is one which has attracted considerable interest. Green and Ryan (1972) showed that wounding of the leaves of tomato induced the rapid accumulation throughout the above-ground plant tissues of a protein which inhibits trypsin and chymotrypsin. They suggested that the formation of proteinase inhibitors following damage was probably a defence, though little is known of their ecological consequences. Haukioja and his colleagues in Finland showed that the growth of larvae of the geometrid moth **Epirrita autumnata** was adversely affected if they were fed either previously grazed leaves of **Betula pubescens** ssp. **tortuosa**, or nearby leaves on the same plant (Haukioja & Niemela, 1977). Later work revealed that there are two, apparently independent, damage-induced responses in the leaves of birch: one is rapidly induced resistance in leaves of the current year, and the other is a delayed resistance which appears in years after defoliation (Haukioja, 1982).

Since these seminal studies many other examples of wound-induced responses in plants have been demonstrated which have been the subject of recent reviews (Edwards & Wratten, 1985; Rhoades, 1979; Fowler & Lawton, 1985). Despite the accumulating evidence of their effects upon insect behaviour or performance, the defensive role of wound-induced changes remains highly controversial. Fowler and Lawton (1985) pointed out that most of the evidence for rapidly induced defences is laboratory based and often reveals relatively small effects upon such things as acceptability of foliage to insects, larvar development rates and pupal weight. They argue that to prove defensive role it must be shown that wound-induced responses can have a significant effect upon herbivore population dynamics in the field, and thereby cause the plants to suffer less damage. They performed a

field experiment with **Betula pubescens** ssp **pubescens** in which trees received different levels of artificial defoliation; however they found no evidence that subsequent insect attack was reduced and concluded that the case for rapidly induced defences in birch remained unproved.

In this paper we argue that wound-induced changes in plant chemistry can have a defensive role against insects, even if there is no detectable effect upon insect population dynamics or upon the total level of grazing damage. We propose a model in which the most significant influence of the induced response is upon the distribution of grazing within the canopy. However first we review the evidence that wounding of foliage can affect subsequent food selection by insects.

2. Wound-induced responses and insect feeding

In many plant species wounding of foliage leads to a reduction in its acceptability to insects. For example in bioassay experiments with birch foliage (**Betula pubescens** ssp **pubescens** and **B. pendula**), using the larvae of **Spodoptera littoralis** and **Orgyia antiqua** as test animals, a reduction in acceptability of both damaged leaves and those nearby on the same branch was detected within 6h following artificial damage (Wratten et al., 1984). In similar bioassay experiments with tomato foliage (Edwards et al., 1985) and using larvae of **Spodoptera littoralis**, grazing levels on damaged leaves were significantly lower than those of the control; within 8h of applying the damage, and within 24h on other leaves of the same plant. These effects persisted for at least 7 days. Leaves from damaged plants commonly showed numerous small holes where feeding had been attempted, and in some experiments grazing on leaves from damaged plants was only about 10% of that of control plants. Using the same basic method we have surveyed a range of British trees (Edwards et al., 1986), and demonstrated wound-induced reductions in leaf acceptability in several species including **Sambucus nigra**, **Crataegus monogyna**, **Corylus avellana**, and **Alnus glutinosa**.

In all these experiments, and in most other studies of induced responses, a relatively high level of artificial damage was inflicted on the foliage. The treatment therefore simulates the level of wounding which might occur during a heavy outbreak of an insect species. In more recent experiments we have examined the effect upon leaf acceptability of very small amounts of damage equivalent to that which a single insect might make during a feeding bout. The question we have investigated, therefore, is: what effect does a single insect have upon its immediate food resources? The experimental damage was made by punching a 5 mm diameter hole on one side of a leaf; widely scattered leaves within the canopy of a tree were chosen to minimise the induction of chemical changes between leaves. After an interval ranging from 0h to 14 days the leaves were picked and placed singly in 9 cm diameter petri dishes with damp filter paper on the top and bottom. One second or third instar larva of **Spodoptera littoralis** was added and the dishes were left at 24°C for 24h. To provide a control against which the influence of the damage upon grazing could be assessed, a second hole was punched in the equivalent position on the opposite side of the leaf after the grazing bioassay was complete.

Figure 1 shows the results of such an experiment on alder leaves (**Alnus glutinosa**) which were picked for bioassay 48h after the damage was inflicted. The figure has been generated using a Kontron IBAS 2 image analysing computer by superimposing images of the 40 grazed leaves used in the experiment. In Fig. 1a the image is centred on the original hole, while in Fig. 1b it is centred on the hole punched as a control. These images thus provided a contour map showing the probability of a particular area of leaf being grazed. The influence of the damage upon feeding by the larvae can be clearly seen as a zone of low grazing in the vicinity of the hole in Fig. 1a.

Gibberd, Edwards and Wratten (unpublished) conducted a series of similar experiments (though not using the image analysing computer) with the leaves of **Alnus glutinosa, Betula pubescens** and **Crataegus monogyna.** The bioassay revealed a reduction in the acceptability of the damaged side of the leaf within 6h for **C. monogyna** and within 24h for the other two species; this effect persisted for at least 7 days.

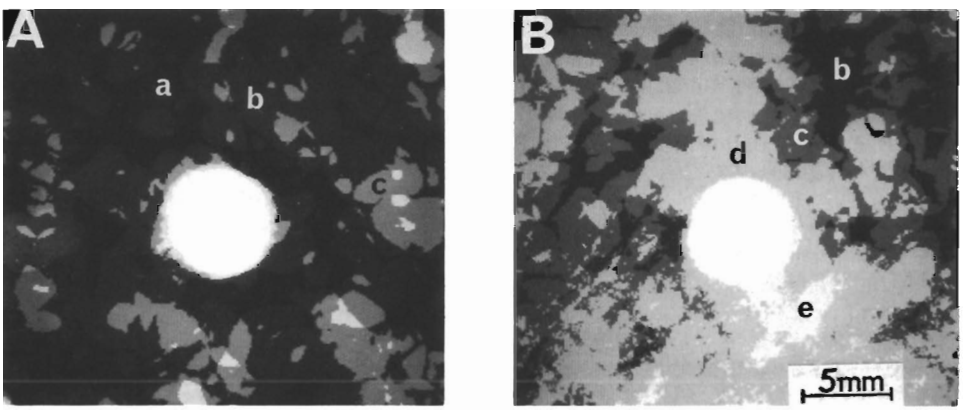

Figure 1. Effect of single holes punched in leaves of **Alnus glutinosa** upon distribution of feeding by larvae od **Spodoptera littoralis.** (A) damaged side of leaf. (B) control side. The shade of grey indicates the percentage of leaves grazed, as follows: 0(a); <5(b); 5<10(c); 10<20(d); 20<30(e).

Silkstone (unpublished) performed an experiment in which one or three holes 8 mm in diameter (representing approximately 5% and 15% respectively of leaf area) were punched in dispersed leaves on trees of **Betula pubescens** and **B. pendula.** The leaves were left on the trees until the end of the season when the percentage of leaf area subsequently removed by insects was recorded. In both species significantly fewer ($P = 0.001$) of the damaged leaves received subsequent grazing than undamaged controls; for example on **B. pendula** (pooling data for the high and low damage treatments) 63% of damaged leaves were grazed compared with 84% of the control. In addition the mean amount of damage to leaves which had received some natural grazing was significantly lower in those which had been experimentally damaged.

The general conclusion to emerge from these experiments is that physical damage to a leaf often reduces either the probability of any subsequent insect grazing, or the amount of grazing if it is attacked. The rate of induction, the duration, and the spatial extent of such effects vary widely between plant species. In very few cases is there any direct evidence that the insects are responding to changes in leaf secondary chemistry though we can eliminate some alternative hypotheses. For example, it has been suggested that insects move away from feeding sites to reduce the chances of capture by predators which seek their prey through observing the leaf damage they make. We do not think this kind of behaviour can explain our results, since we find that the influence of a hole in a leaf upon insect feeding changes with the interval since damage. It may be that leaf damage leads to increased water loss and insects avoid the tissues with reduced hydration. However this effect would not help us explain reduced acceptability of leaves adjacent to those damaged. In any case, when we measured the water content of leaf tissue surrounding single holes in the leaves of **Betula pubescens** 48h after they had been punched we did not detect any difference compared with undamaged leaf tissue.

3. The defensive role of rapidly induced responses

Table 1 presents two models of how rapid wound-induced changes in leaf chemistry might protect a plant. According to the first model changes in secondary chemistry are induced in all foliage above a certain level of damage, and the plant is thereafter more resistant to insect herbivores. As a result the insect population is adversely affected and the plant sufffers less subsequent damage from insects. Because there is a threshold of damage above which the chemical changes occur we would expect such a defence to become effective following an outbreak of an insect population, and for much of the time it would not operate. This is essentially the model tested by Fowler and Lawton (1985) in their experiment in which they damaged the foliage of **Betula pubescens** and then studied the accumulation of natural grazing damage. In that experiment there was no evidence that even high levels of damage (25% defoliation) affected the subsequent level of insect grazing. However, we do no dismiss this model, which may apply in certain circumstances, particularly in the case of delayed induced resistance (Haukioja & Hakala, 1975; Haukioja, this symposium).

In model 2 there is no threshold, and any damage to leaves, however small, causes at least local changes in leaf chemistry. An insect responds to the induced change by moving away from the vicinity of damage; the result for the plant is a distribution of grazing damage, both within and between leaves, which is more dispersed than it otherwise would be. This step in the model is supported by wealth of evidence from the kinds of experiment described above, showing that insects tend to feed some distance away form previous damage. In general our experiments show that this deflection away from damage is strongest in young leaves.

How could alteration ot the distribution of grazing damage act as a defence? The plant may benefit because insects spend more time foraging for suitable tissues. In addition mortality risks through predation or falling off the plant may be greater for an insect which must continually move

(Schultz, 1983). However we suggest that the most important influence of the distribution of grazing damage is upon a plant's success in competition.

For plants growing in highly productive habitats the most important biotic factors are neighbouring plants with which they must compete for resources. If water and nutrient conditions are adequate then light is the principal limiting factor, and competition takes the form of a continuous struggle to occupy the outermost canopy. In competitive conditions the outermost leaves, which are mainly the new leaves, are the most important to the plant for successful competition, and plants characteristic of such environments tend to produce new leaves throughout much of the growing season as they 'forage' for light (Grime, 1979). However young leaves are likely to be most vulnerable to insects since they are less tough and have higher nitrogen concentrations than older leaves. For insects, young leaves on a plant with continuous extension growth are a predictable resource of high quality food. We suggest that the significance of rapid wound-induced responses may be that they minimise damage to young leaves by deflecting grazers away from them. Support for this hypothesis comes from the range of species in which we can detect a rapidly induced reduction in palatability (Edwards & Wratten, 1985). Species such as **Crataegus monogyna, Betula pubescens** ssp **pubescens** and **Alnus glutinosa** are plants of competitive, early successional habitats and exhibit leaf production over much of the growing season ('competitors' sensu Grime, 1979). In contrast we have not detected rapid responses in trees such as **Quercus robur** and **Tilia cordata** which are more characteristic of climax woodland and which have much shorter periods of leaf production.

Table 1. Contrasting models for the defensive role of wound-induced chemical changes in plants.

Consequences of grazing	Model 1	Model 2
1. For foliage	above a certain threshold of damage chemical changes are induced in all foliage; an off/on process	any level of grazing induces at least local changes; a continuous process.
2. For individual herbivore	growth, survival and fecundity suffer	main effect is movement away from site of feeding: performance effects secondary
3. For herbivore population dynamics	significant reduction of population levels	reduction in population may or may not occur
4. For plant	plant protected: less overall damage from subsequent grazing	grazing damage dispersed, especially away from young leaves: overall effects secondary

A major gap in the avalaible evidence presented here for model 2 is the experimental demonstration that the distribution of grazing damage does not affect the outcome of competition. We believe that future work on plant defences must consider the infuence of insect grazing upon the competitive relations of plants, especially in relation to selective grazing of young plant tissues.

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CHAPTER 5. INFLUENCE OF THE HOST PLANT AS CONDITIONING AND SELECTIVE FACTORS OF INSECTS. POPULATION GENETICS IN PLANT-INSECT RELATIONSHIPS.

Labeyrie, V., Fabres, G., Lachaise, D. (eds) Insects – Plants

POPULATION GENETICS AND FOODPLANT USE AMONG THE NORTH AMERICAN TREE-FEEDING PAPILIONIDAE

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1. Introduction

The North American swallowtail butterflies of the genus **Papilio** are among the most polyphagous of all 563 species of Papilionidae in the world (Scriber, 1984a). The tiger swallowtail **Papilio glaucus** L. is particularly noteworthy in this regard as it has the widest range of recorded host plants of any species, however local specialization is known to occur (see Scriber, 1986a). The process by which host choices are made by insects have been the theme of this symposium series for decades, however the genetic bases of these interactions has remained largely without serious attention. Perhaps we may be unable to adequately address aspects of genotypic regulation of insect-plant interactions until we better understand the various environmental factors affecting host choice and host suitability (Scriber, 1984b; Maddox & Cappuccino, 1986). While the underlying genetic basis is implicit in discussions of adaptations, counter-adaptations, and coevolution between insects and their plant hosts, a continuing dearth of genetic studies persists (Dethier, 1978; Gould, 1983; Mitter & Futuyma, 1983).

The genetic basis of chemically mediated interactions between insects and their hosts does not necessarily involve coevolutionary responses and it is important that precise definitions of terms be employed to avoid confusion in this regard (see Diehl & Bush, 1984; Pyke, 1984; Futuyma & Peterson, 1985; Gould, 1986). Even the non-genetic aspects of host "preference" and "suitability" may require revised terminology and thought (Mattson & Scriber, 1986; Singer, 1986). Finally, our ability to distinguish between genetic and non-genetic factors affecting host selection and/or suitability may be complicated by a number of things (including learning and non-genetic inheritance of preferences; see Corbet, 1985; Hoffman, 1985; Courtney, 1986; Gould, 1986; Papaj, 1986).

Little is known about the degree of genetic correlation between **Papilio** host selection and host suitability, although oviposition "mistakes" (i.e. on plants which are toxic to larvae) are known to occur (Brower, 1985; Scriber, 1973; Wiklund, 1975; Berenbaum, 1981; Feeny et al., 1983). The general occurrence and extent of coadapted preference/viability gene complexes in insects with regard to host selection and utilization is surprisingly poorly known in spite of its obvious significance in basic and applied fields (Gould, 1983; Diehl & Bush, 1984; Service, 1984; Futuyma & Peterson, 1985; Via, 1986). Genetic variation in herbivores for host plant selection (Tabashnik et al., 1981; Jaenike & Grimaldi, 1983; Singer, 1983;

Futuyma et al., 1984; Jaenike, 1985) and for larval performance (Tabashnik, 1983; Rausher, 1984; Via, 1984; Scriber, 1986a) are known to exist, but little is known about the genetic covariance between preference and performance (see Futuyma & Peterson, 1985).

Another topic of continuing general interest concerns the concept of feeding specialization and associated adaptation (behavioral and biochemical). The implication from ecological literature that "a jack-of-all-trades is a master of none" has been a central theme in our research with the **Papilio** (Scriber & Feeny, 1979; Scriber, 1983, 1986c). Of particular concern is whether trade-offs will result from adaptation to one host or host defense with regard to fitness on a second host or defense (see also Gould, 1979; Rausher, 1983, 1984; Via, 1984a). Most of our studies to date have concerned the significance of variation in plant quality for the insect (Scriber, 1977; Scriber & Slansky, 1981; Scriber, 1984c) since these non-genetic aspects of larval survival, growth and final size can largely obscure the genetic (heritable) traits of the insect performance (e.g. wing size and pupal weights in **Papilio glaucus** in relation to leaf quality; see Scriber, 1982; Via & lande, 1985). Also of significant concern in assessing the variation in larval performance will be the previous experience of the population and of the individuals in the population (e.g. Grabstein & Scriber, 1982; Scriber, 1981, 1982; Hoffman, 1985).

This report will outline the general approach we have taken in attempts to understand the evolution of foodplant use patterns among the North American tree-feeding swallowtails of the P**apilio glaucus** group (**P. g. glaucus** L., **P. g. canadensis** R & J, **P. g. australis** Maynard, **P. rutulus** Lucas, **P. eurymedon** Lucas, **P. multicaudatus** Kirby, and P. **alexiares** Hopffer) and the **Papilio troilus** group (**P. troilus** L., **P. palamedes** Drury, and P. **pilumnus** Boisduval). The significance of these differential foodplant use abilities upon the biogeographical distribution and systematic relationships among various taxa are discussed elsewhere (Scriber, 1986b, 1986c). Significant intrasubspecific differences exist among different geographic populations of **papilio** (e.g. Scriber, 1983), but can not be elaborated upon here.

2. Procedure

2.1. Insect cultures; collection, handpairing, and mass-rearing

Adult female butterflies from across North America were returned to the laboratory for oviposition on acceptable foodplant leaves supported in water-filled aquapics in clear plastic boxes (12 cm x 20 cm x 30 cm) under heat from an incandescent lightbulb placed at a distance approximately 0.5 meter from the boxes. Eggs were removed on leaves from the boxes and neonate larvae were subsequently distributed to various foodplants in controlled environment conditions where they were reared through to pupation and eclosion as adults. Hand-pairings of lab-reared virgin females with lab and/or field captured males were conducted in the laboratory, with subsequent treatment of the females and offspring as described above.

2.2. Foodplants

Plant species for feeding bioassays of neonate larval survival and growth performance were obtained from Dane County, Wisconsin except for 1) balsam poplar, **Populus balsamifera** L. which was collected in northern Wisconsin and Michigan, 2) sweetbay, **Magnolia virginiana** L., which was obtained from Florida and Texas (as well as the University of Wisconsin Arboretum) and 3) red bay, **Persea borbonia** (L.) Spreng., which was also form Florida and Texas. Additional bioassays of a hundred and thirty additional plant species (including some from the Pacific coast are described elsewhere, Scriber, 1986c).

3. Patterns of foodplant use

3.1. Local Preferences and Selection Pressures

Within a given plant community, a variety or mosaic of potentially suitable host plant choices will be available, however a variety of behavioral, biochemical, or ecological selection pressures can significantly reduce the realized range of plant species actually selected by a herbivore (see reviews in Fox & Morrow, 1981; Scriber, 1983). With selection pressure for use of one plant species or genotype (e.g. plant "D" in Fig. 1), a number of outcomes are possible regarding the ability to successfully utilize the others. Among these is the possibility that there will be no change in the herbivore fitness on plant "D". Alternatively if selection for plant "D" results in an increased preference/fitness, several additional variations are possible: 1) no change in preference/fitness will be observed on the other species (i.e. A, B, or C); 2) an increase in fitness will be observed on the others; 3) a decrease in fitness will be observed on others (i.e. negative genetic correlations or "trade-offs"); 4) or variable responses will be observed with preference/fitness increased on some, decreases on others, or unaltered by the selection for use of "D" (Fig. 1; cf also Gould, 1986).

These local adaptations and apparent trade-offs in larval preference, performance, on selected locally preferred hosts relative to other potential hosts are known to occur at an intraspecific and intrasubspecific level (e.g. Scriber, 1983, 1986a, b). Most significant however are some of the interspecific differences in foodplant use among the North American **Papilio glaucus** and **P. troilus** groups (see Fig. 2; Scriber, 1986c).

3.2. Neonate larval survival: a comparison

Plant species from eight different families were bioassayed using neonate (newly eclosed) larvae from 10 different **Papilio** taxa including all of the **P. glaucus** group, all but **P. pilumnus** of the **P. troilus** group, and **P. cresphontes** (Fig. 3). Blank spaces indicate no larval survival on the plant species, and it is apparent that **P. troilus** and **P. palamedes** survived only on the Lauraceae. Similarly, **P. crephontes** survived only on the Rutaceae. However, the various taxa of the **P. glaucus** group all had some larvae survive on the Rutaceae and Rosaceae, and even some on the Lauraceae (Fig. 3).

Two basic groups of three taxa each appear to separate with regard to their abilities to survive on the Salicaceae and the Magnoliaceae (in the

south and eastern part of the continent, **P. alexiares**, **P. g. glaucus**, and **P. g. australis** do poorly on quaking aspen of the Salicaceae; while **P. g. canadensis**, **P. rutulus** and **P. eurymedon** of the north and west do poorly on the Magnoliaceae species tested). Further specialization of **P. rutulus** on the Plantanaceae in California and of **P. eurymedon** on the Rhamnaceae is reflected in relatively high survival of their neonates relative to all of the other butterfly taxa tested.

3.3. Genetic bases of differential survival

Hand-paired F_1 hybrids between various taxa have been conducted in an attempt to determine if the differences in detoxication abilities are heritable. Several thousand crosses during the last 4 years have heen helpful in determining that these differences do indeed have a genetic basis (see Scriber, 1986a, 1986c). Of particular significance are the differences in ability for utilizing the Magnoliaceae and the Salicaceae (Fig. 3) and the capability of transferring these abilities through the hybrids and backcrosses. In essentially all (>90%) of our laboratory hybrids, we observe excellent survival of hybrid individuals (from a single brood/mother) on both tulip tree, **Liriodendron tulipfera**, and quaking aspen, **Populus tremuloides**, as well as on black cherry, **Prunus serotina** (Table 1). Black cherry serves as a general common denominator with regard to suitability, and therefore is useful as a control for assessment of F_2 breakdown ("hybrid dysgenesis") and interpretation of backcross studies (Scriber, 1986a). While not indicated in table 1, **Papilio eurymedon** survival parallels that of **rutulus** on these three foodplants and the various crosses also reflect a virtually identical parallel pattern (e.g. **P. g. glaucus** x **P. eurymedon** hybrids exhibit 64% survival on tulip tree). Also, **eurymedon** hybrids with **P. rutulus** and with **P. g. canadensis** exhibit absolutely no ability to use tulip tree leaves (survival is 0% in all cases; as it is with pure **eurymedon** stock). Conversely, **P. eurymedon** can survive well on quaking aspen and this ability is transferred to hybrid larvae (e.g. **glaucus** x **eurymedon**).

As Gould (1986) points out, if we want to make predictions about what determines the observed host range patterns of herbivores, we can not simply look at the plant-specific performances in a single herbivore species population. We must assess various populations and their genetically correlated adaptations. This is where a reductionistic (mechanistic/biochemical/process-oriented) approach becomes most useful at understanding the operational aspects of adaptations. Our results with neonate **Papilio** survival and penultimate/final instar growth bioassays suggest that the mode of antibiosis in the Magnoliaceae and Salicaceae is primarily a major metabolic expense (rather than a feeding deterrent or digestibility reducer). In addition to a series of extensive biogeographic bioassays, we hope to eventually work out the phytochemistry (Lindroth et al., 1986; Scriber et al., 1986) and enzyme genetics responsible for the detoxication abilities of these remarkable hybrids and backcrosses of **Papilio** taxa.

Figure 1.

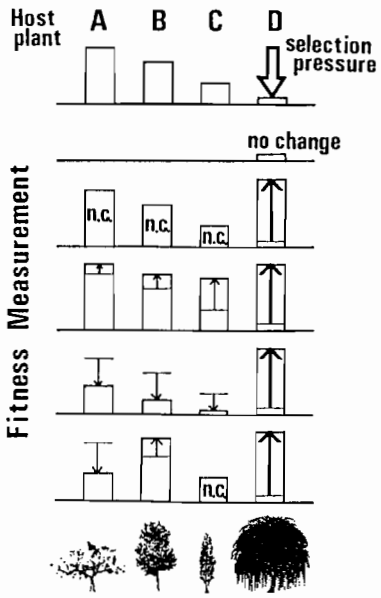

Figure 2.

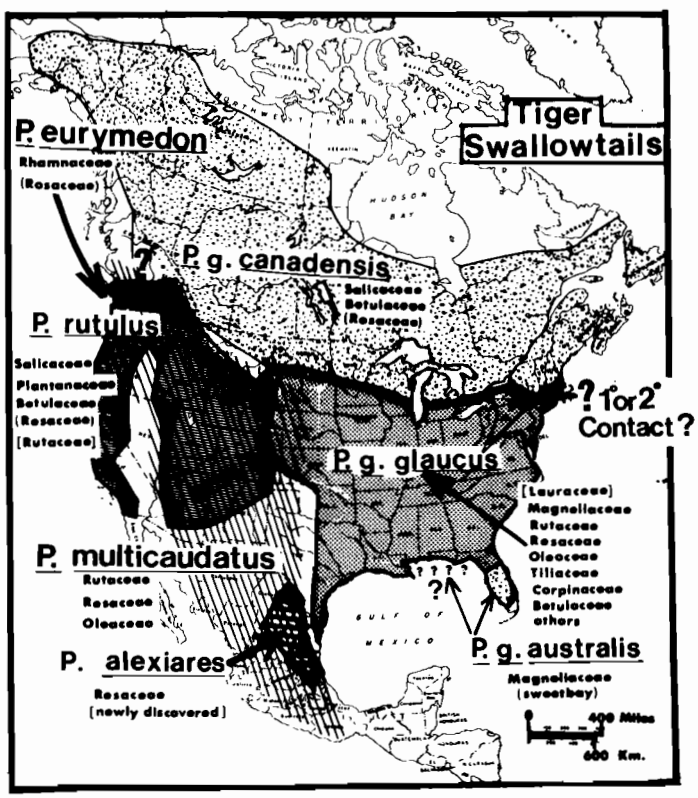

Table 1. Survival percentage and number of neonates tested (in brackets).

Parents & hybrids	No. of mothers	Food plant: Quaking aspen	Black cherry	Tulip tree
P. rutulus (r)	6	68 (38)	87 (30)	0 (10)
P. g. canadensis (c)	206	79 (358)	74 (2872)	1 (420)
cxr	11	69 (52)	66 (100)	0 (46)
gxr	16	66 (190)	79 (434)	65 (234)
axc	4	94 (46)	76 (109)	66 (92)
cxa	1	-	-	100 (2)
cxg	10	78 (88)	84 (117)	76 (129)
gxc	91	61 (690)	80 (3020)	75 (885)
axr	9	50 (96)	69 (129)	84 (147)
gxa	2	11 (9)	47 (15)	71 (7)
axg	2	0 (8)	64 (11)	90 (10)
P. g. australis (a)	31	6 (124)	83 (304)	75 (276)
P. g. glaucus (g)	207	7 (2101)	80 (4564)	80 (2085)

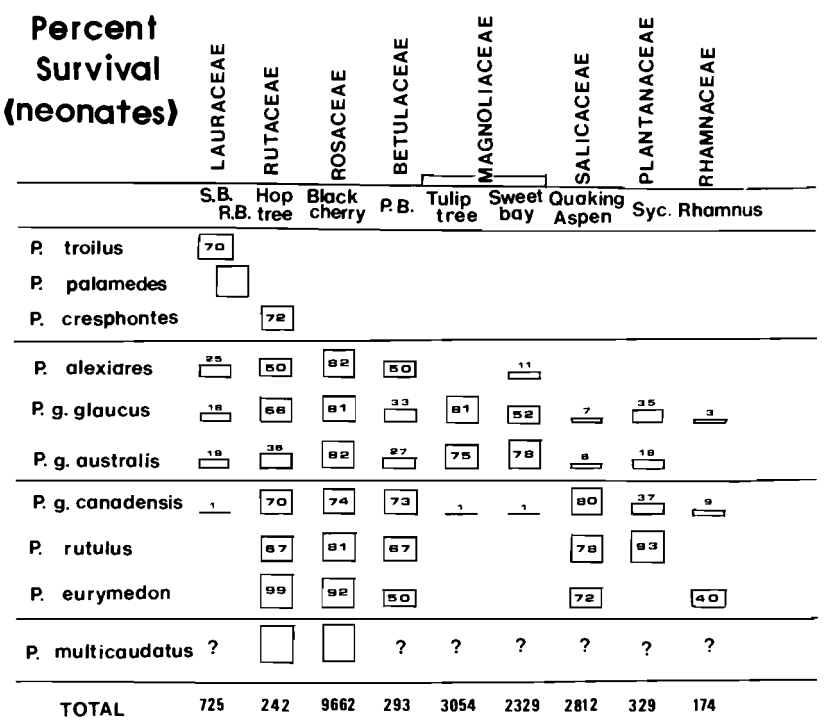

Figure 3. S.B. = sweetbay, **Magnolia virginiana**; R.B. = red bay, **Persea borbonia**; P.B. = paper birch, **Betula papyrifera**; Syc. = Sycamore, **Platanus.**

4. Conclusions

Increased attention is warranted with regard to the genetic aspects of insect-plant interactions, especially since so little is currently known

and so much is otherwise left to presumed ecological wisdom and assumptions.

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THE ROLE OF HABITUATION IN FOOD SELECTION OF LEPIDOPTEROUS LARVAE: THE EXAMPLE OF MAMESTRA BRASSICAE L. (LEPID. NOCTUIDAE).

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1. Introduction

Experience may modify the feeding behaviour of phytophagous insects (see Jermy, 1986 for ref.). Habituation as defined by Groves and Thompson (1970) has been found to occur in case of feeding inhibitors mostly with polyphagous species (Jermy et al., 1982; Szentesi & Bernays, 1984). The latter studies were carried out among others with the polyphagous **M. brassicae** using single allelochemicals as feeding deterrents. Since deterrency is generally due to several substances present in the plant (Jermy, 1983), the question arises whether polyphagous insects are able to habituate to deterrent plants, i.e. whether they habituate to a complex of deterrent stimuli and so may extend their host plant ranges.

2. Material and methods

M. brassicae was reared at 27 ± 1°C on a semisynthetic diet (Nagy, 1970). Freshly ecdysed 6th instar larvae were used for the experiments that were carried out at 23 ± 1°C and 16/8 hr L/D phototoperiod (scotophase 20.00 to 04.00). Illumination was ca 15 Lux. The plants were taken from the field except the **Solanum** spp. that were grown in a glass house. The data were analysed by the Duncan's test for unequal sample size.

2.1. Detterency test

Freshly ecdysed larvae were given cabbage **ad libitum** at 09.00. At 14.00 they were weighed and arranged in weight classes. At 16.00 the larvae were put singly in 100 ml plastic cups that were covered by a glass sheet. A piece of wet filter paper kept air humidity high. Disks (15 mm diam.) were punched from the leaves. For each cup a certain number of disks was taken and their fresh weight was measured. For each plant species 10 to 20 larvae were randomly taken from the weight classes so that all classes were represented in each treatment. The larvae fed on the disks from 16.00 to 09.00, then were removed. The area consumed from each disk was estimated by sight and the fresh weight consumed was calculated (accuracy: ± 5%). The degree of deterrency was calcutated as follows:
$I = 100 - NP/HP \times 100$, where NP = average amount consumed from the non-host plant, HP = average amount consumed from cabbage in the same experiment.

2.2. Habituation experiments

Two subsequent experiments were carried out:

2.2.1. Daily exposure test. On day 1 the same procedure was followed as in 2.1. On day 2 at 09.00 the larvae were not removed but were given cabbage **ad libitum**. At 16.00 a new cycle began. This was repeated until the larvae finished feeding. The nightly consumption was measured.

2.2.2. Comparison test (Fig. 1). 19 larvae were treated as in 2.2.1 and were called "experienced" (E). A large number of larvae kept also individually formed the "stock" (S): they were fed from 09.00 to 16.00 with cabbage **ad libitum** (HP-A) and from 16.00 to 09.00 with a limited amount of cabbage that equalled to the amount consumed from the non-host plant disks (NH) by the larvae on the same days of the instar in the preceding 2.2.1 test. Each day at 16.00 15 "naive" (N) larvae were taken from S and given NH-disks. The consumption by both E and N larvae from the NH-disks was measured.

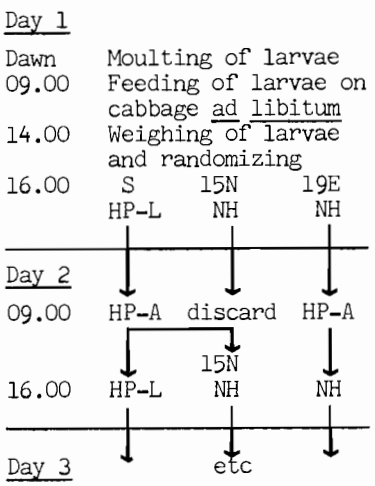


Figure 1. Design of the comparison test. S = "stock", N = "naive", E = experienced larve. HP-A = cabbage **ad lib.** HP-L = limited amount of cabbage. NH = non-host plant disks.

3. Results and discussion

The deterrency test was carried out with 47 plant species from 25 families. Interestingly, 55% of the species showed more than 90% deterrency.

The degree of deterrency and the distribution of the larvae among three feeding classes are shown for 20 species in Table I. There was striking variation in the amounts consumed by individual larvae, especially with plants showing about 90% deterrency. In extreme cases (e.g. **S. tuberosum**) the smallest and the greatest amounts consumed on the first day exceeded the ratio of 1:200! Much less variation occurred with cabbage.

In the daily exposure tests there was no correlation whatever between the amounts consumed by an individual larva from the non-host plant and the host plant, and between the amounts consumed from the non-host plant and the weight of the larva. Thus, variability in the acceptance of a non-host

plant is due most probably to behavioural (sensory) and not to physiological differences.

The average amounts consumed by the larvae from the non-host plants through the instar gave the following main patterns:

a) The amounts consumed daily did not change significantly through the instar in case of the plant species Nos 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 (see species names in Table I). Thus, no habituation occurred with these plants. Consumption was from the beginning on so reduced that an eventual decrease of acceptability remained unapparent.

b) With **H. helix** there was a sudden significant (P = 1%) increase of consumption on the 2nd day followed by a significant (P = 1%) reduction (Fig. 2). The increase on the 2nd day was considerably greater (1:3.3 for 1-yr-leaf, 1:3.7 for 2-yr-leaf) than with the host plant (1:1.5) on the same day. **P. quinquefolia** caused a similar effect. The sudden increase of consumption may suggest habituation, while the sharp decrease hereafter may indicate aversion learning evoked by some adverse effect cause by the large amounts consumed from the non-host plant on the 2nd day, although no symptoms of illness were apparent.

c) With the other plant species the pattern of daily consumption tended to decrease (Nos 13, 18, 19), increase (12, 15, 17) or remained more or less at the same level (14) (see species names in Table I) through the instar. Since the enormous individual variability may have masked subtle behavioural changes, the larvae were arranged arbitrarily in three groups according to the relative amounts consumed from the test plant through the instar, namely, in groups of "poor", "medium", and "gross eaters" and the data were evaluated accordingly.

Table I. Degree of deterrency and distribution of larvae among three feeding classes (amounts consumed on Days 1 to 5).

No.	Plant species	Deterr. %	% larvae consuming 0-100 mg	100-200 mg	>200 mg
1	Trifolium montanum	100	100	-	-
2	" alpestre	100	100	-	-
3	Solanum americanum	99	100	-	-
4	" luteum	99	100	-	-
5	" nigrum	99	100	-	-
6	" alatum	99	100	-	-
7	" nodiflorum	99	100	-	-
8	" macroglobularum	99	100	-	-
9	" paranense	99	100	-	-
10	" pseudocapsicum	99	92	8	-
11	Alliaria officinalis	97	80	20	-
12	Ligustrum vulgare	97	50	50	-
13	Cynanchum vincetoxicum	96	90	10	-
14	Syringa vulgaris	94	73	27	-
15	Dictamnus albus	94	22	11	67
16	Parthenocissus quinquefolia	93	10	30	60
17	Solanum saponaceum	88	7	13	80
18	Philadelphus coronarius	85	20	10	70
19	Solanum tuberosum	83	17	11	72
20	Hedera helix /2-yr-leaf/	71	-	-	100
	" " /1-yr-leaf/	50	-	-	100

Figure 2. Amounts consumed by **M. brassicae** larvae from young (A) and old (B) leaves of **Hedera helix** during the daily exposure test.

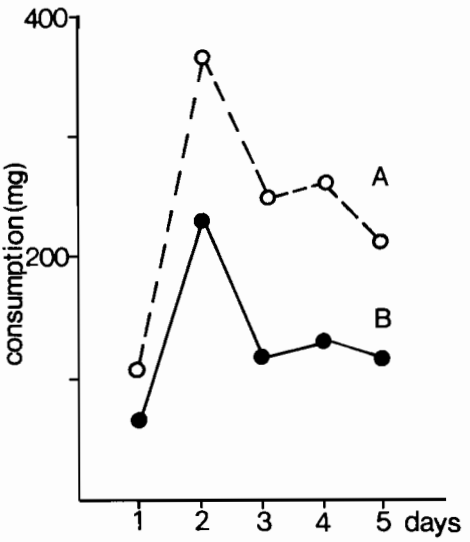


Figure 3. Comparison test with **M. brassicae** larvae. Test plant: **Solanum tuberosum.** One asterisk = sign. diff. ar 1%, two asterisks = sign. diff. at 5% level from "naives".

Figure 4. Comparison test with pretested **M. brassicae** larvae. Test plant: **Solanum tuberosum.** Asterisks mean significant differences from "naives" at 1% level.

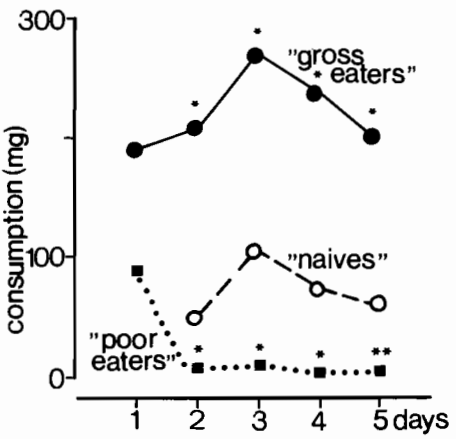
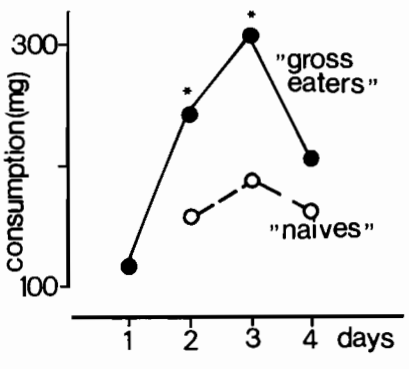
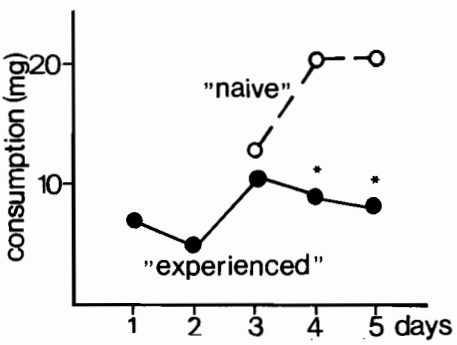


Figure 5. Comparison test with **M. brassicae** larvae. Test Plant: **Syringa vulgaris.** Asterisks mean significant differences at 1% level.





In case of **S. tuberosum** (Fig. 3) the "gross eaters" habituated while the "poor eaters" showed aversion learning of kept eating very small amounts through the instar. With only "gross eaters" that were sorted out by a pretest, temporary habituation occurred (Fig. 4). The "gross eaters", however, did not exceed 10% of the larval population, and the decrease of consumption was restricted to "the poor eaters" (ca 20% of the population) only, thus, the bulk of the population did not change its feeding behaviour.

With **S. vulgaris** (Fig. 5) instead of habituation a tendency to aversion learning has been found.

Neither with the plant species listed under a), nor in the above cases when a significant decrease of acceptance has been demonstrated, suggesting aversion learning, did the larvae show symptoms of illness, contrary to Dethier's (1980) finding in two polyphagous lepidopterous species. It is, therefore, doubtful whether the two phenomena have identical physiological and/or behavioural backgrounds. Blaney et al. (1985) proposed a mechanism of short-term learning to explain the rejection of unpalatable plants by locusts. By analogy, the increasing rejection of non-host plants by **M. brassicae** may reflect lon-term learning that is not wiped out by intermittent feeding on the host plant. Most probably both short- and long-term learning processes resulting in the increase of rejection, are due to sensitization, the counterpart of habituation (Groves & Thompson, 1970).

In conclusion, the larvae of **M. brassicae** are less able to habituate to the deterrent stimuli of the non-host plants contrary to the relative easiness of habituation to single plant chemicals (Jermy et al., 1982). Supposedly this is due to the multiplicity of deterrent chemicals in plants. If this turns out to be characteristic also of other species, it would suggest, that both in plant breeding for resistance based on non-preference and in search for feasible antifeedants striving for complexity of deterrent stimuli may be more promising than dealing with single compounds only. Further studies are necessary to reveal the possible genetic basis of the extreme individual variability in feeding behaviour. If it is genetically determined, even the small fraction of the population that is able to habituate, might initiate the appearance of strains with novel feeding habits. Since the population of **M. brassicae** used in the above experiments has been inbred for more than 100 generations, its genetic homogeneity is likely. It may indicate that in this case behavioural variability is due to phenotypic differences only.

Abstract

Non-host plants were tested for degree of deterrency by sixth instar **M. brassicae** larvae. The larvae showed extreme individual variability in the acceptance of plants. Repeated exposure to a non-host plant did not change its acceptability by the bulk of the larval population, however, in some case "gross eaters" (<10% of population) tended to habituate while with "poor eaters" (ca 20% of population) acceptance decreased. It is supposed that larvae are unable to habituate to the multiplicity of feeding inhibitors present in the plants.

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EXPERIENCE: A MODIFIER OF NEURAL AND BEHAVIOURAL SENSITIVITY

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It is well known that there is wide variation in food selection by insects. In part, this is due to apparently random variability between individuals and it differs in magnitude with different species, polyphagous species often showing greater variability than oligophagous species (Simmonds & Blaney, 1984; Wiklund, 1981). Alternatively, variation in response may be related to pretreatment of the insects. When the effect is short-term, it is described as learning (Blaney & Simmonds, 1985; Traynier, 1985) and when a longer time scale is involved, the term induction has been used (Jermy et al., 1968; Blaney & Simmonds, 1984; De Boer & Hanson, 1984). We have investigated these effects on larvae of **Spodoptera littoralis** and **S. exempta**, in relation to diet, age in the instar and time of day.

The larvae were reared at Birkbeck College under conditions previously described (Simmonds & Blaney, 1984). **S. exempta** were reared on wheat and **S. littoralis** on a bean-based artificial diet. To investigate the effect of recent experience, 6th instar larvae of **S. exempta**, deprived of food for 2 hr were allowed six successive contacts with potential food material in a Petri dish at 10 min. intervals and details of the behaviour of individual larvae were recorded. In assessing the food, the larvae used a hierarchical test sequence similar to that described for locusts (Blaney et al., 1985). The larva palpates (P) on the surface with maxillary palps and stylocomic sensilla; bites (B) with a single bite; nibbles (N) taking several bites without ingesting; samples (S) by taking in a small piece of food; and finally feeds (F). Rejection can occur at any stage prior to feeding. With the favoured food, wheat, most larvae went on to feed at the first contact but some rejection did occur (Fig. 1).

Analysis of the behaviour of individuals showed that those rejecting initially went on to feed subsequently and by the third contact all larvae had fed. If a non-favoured plant, cotton, was presented, the stage at which rejection occurred changed with successive contacts (Fig. 2). Initially most larvae tested the leaf surface by palpation, then went on to test the internal contents as well before rejecting. On later contacts, rejection occurred increasingly earlier in the test sequence and in many cases palpation alone would trigger rejection. Apparently the larvae are learning to associate the sensation experienced after biting, on which rejection is initially based, with that obtained on palpation so that subsequent contacts are terminated at the more preliminary stage in the test sequence. A more detailed argument that similar behaviour in locusts is an example of associative learning is to be found elsewhere (Blaney & Simmonds, 1985).

Thorpe (1963) has argued that forgetting is a necessary concomitant to learning. The larvae might be caused to forget, either by a novel, related experience, or simply by the passage of time. A series of contacts with cotton, at 10 min intervals, was interrupted by two successive contacts with wheat (Fig. 3).

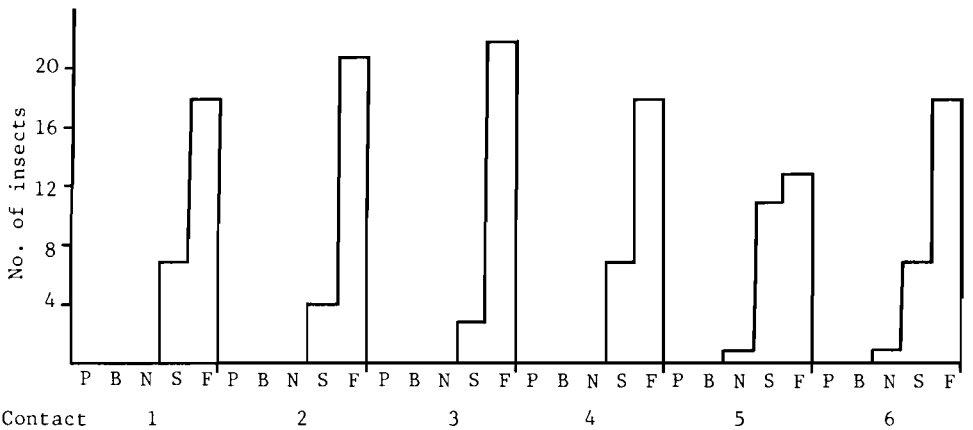

Figure 1. Food selection: the level in the hierarchical test sequence that rejection occurred. Plant: Wheat

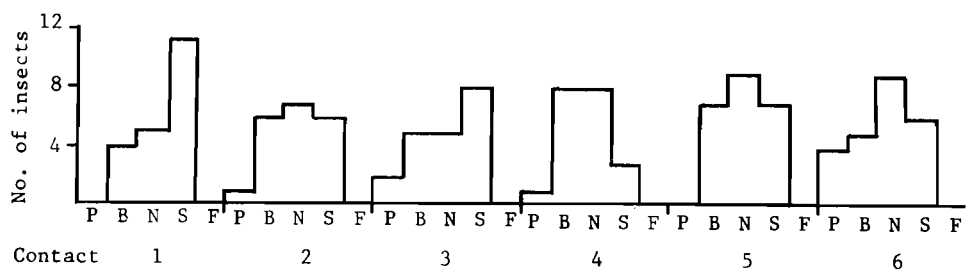

Figure 2. Food selection: the level in the hierarchical test sequence that rejection occurred. Plant: Cotton

The learning process occurred between the first and second contacts with cotton as before but was extinguished by the wheat contacts so that contacts 5 and 6, on cotton, were essentially the same as contacts 1 and 2. When contacts with cotton and wheat were alternated (Fig. 4) there was no evidence of learning. A series of encounters with cotton was presented with 45 min between successive contacts. In this experiment there was some learning associated with the first contact, but the difference between the first two contacts was slight and the pattern of rejection on subsequent contacts did not change. Clearly, the learning involved here is associated with short-term memory and is extinguished by the forgetting occurring during the 45 min interval. Further experiments are being undertaken to

measure the duration of this memory and the results will be presented elsewhere.

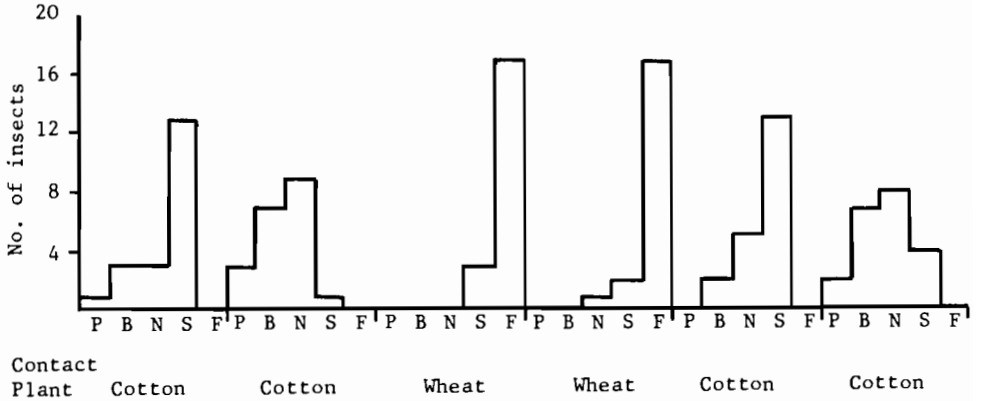

Figure 3. Food selection: the level in the hierarchical test sequence that rejection occurred. Plant: Cotton and Wheat.

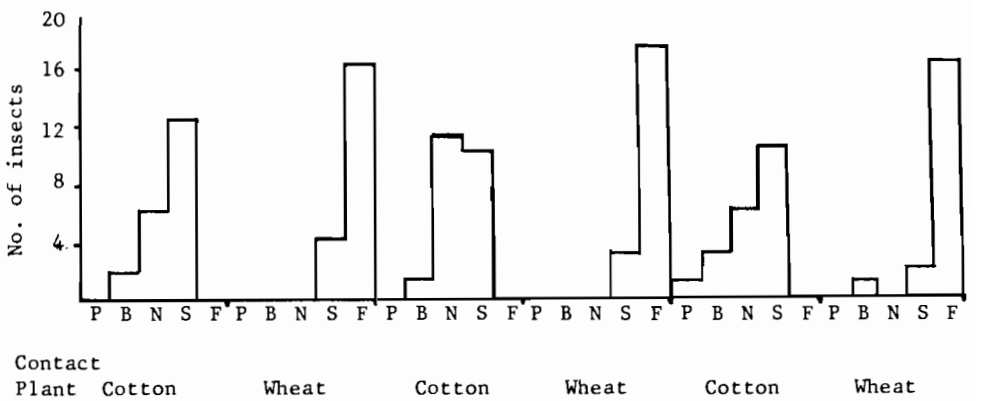

Figure 4. Food selection: the level in the hierarchical test sequence that rejection occurred. Plant: Cotton and Wheat.

Less transient modifications of food preference are associated with changes in diet over a period of time. Such changes have involved alteration of the principal, or only, food plant offered to the insects (de Boer & Hanson, 1984) or alterations to the constituents or artificial diets (Blaney et al., 1986). In either case it is assumed that allelochemicals play an important role. Addition of a novel allelochemical to the diet may enhance the ability of the insect to detoxify allelochemicals in general, or that allelochemical in particular (see McCaffery, this volume). Such increased capacity to avoid the ill effects of an allelochemical would not in itself alter the insect's behavioural response to plants or diets containing the chemical. Nevertheless, behavioural sensitivity does alter so that the insect is prepared to accept more of such food material. We

have investigated this phenomenon, both with artificial diet and with host plants, and have studied the sensory physiology associated with the behavioural change. Larvae of **S. littoralis** were reared on a bean-based artificial diet (AD), following three different regimes: having unmodified diet throughout the larval phase; having plain diet at first, then diet containing 0.02 M nicotine hydrogen tartrate (NHT) from the fourth instar onwards; having diet containing NHT (0.02M) from the first instar onwards. Two further experimental groups were reared on cabbage, **Brassica** sp. (BR) or wheat, **Triticum** sp. (TR) and had either unmodified plant material or, from the fourth instar onwards, plant material sprayed with 0.02 M NHT. The effects of these treatments were assessed in the sixth instar when all larvae were tested with glass fibre discs made palatable with sucrose (0.05 M) and containing NHT (0.02 M). This food material, which is readily eaten (Simmonds et al., 1985) was novel for all the insects and its palatability was assessed by measuring the duration of the first feeding bout (Fig. 5). The naive larvae (N) from all diets (AD, BR, TR), which had never experienced NHT, ate only a small amount whereas those which had NHT in their food from the fourth instar (E_4) ate considerably more. The greatest intake occurred in larvae which had experienced NHT from the first instar onwards (E_1). Thus experience of NHt in the diet results in greater consumption of NHT, and the effect increases with increased exposure to the chemical.

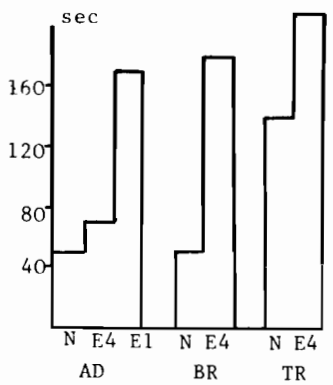

Figure 5. The duration of the first feeding bout.

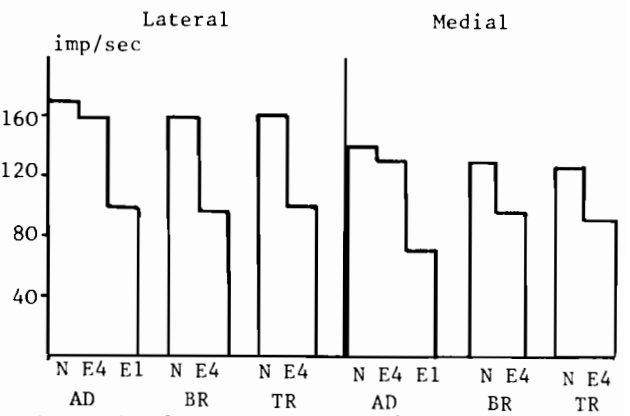

Figure 6. Electrophysiological response of the lateral and medial styloconic sensilla.

Larvae given the same pretreatments were tested in an electrophysiological assay. The input from the maxillary styloconic sensilla is critically important in food selection (see Hanson, this volume) and these sensilla were tested with a solution containing 0.05 M sodium chloride and 0.02 M NHT. The responses (Fig. 6) represent the firing of all the neurones in the lateral and medial sensilla during the first second of stimulation. A substantial part of the response is the activity of 'deterrent' neurones which are sensitive to NHT and whose activity tends to inhibit feeding. Experience of the chemical alters the responsiveness of these sensilla in a way which correlates with the changes in feeding

behaviour observed and is similarly related to the degree of previous exposure. We have obtained similar results with other allelochemicals (in press).

The mechanism by which dietary constituents produce a feedback effect on the functioning of peripheral receptors in unknown and much remains to be done to assess the specificity of the system. Wether the feedback is direct, involving the allelochemicals themselves, or indirect, involving some intermediate such as a hormone, it may reasonably be supposed that the haemolymph is involved, if only as a passive transport system. There is no **a priori** reason to suppose that the system will not be a general one and it may be that nutrients also have an effect. Indeed there is a growing body of evidence to support this (see review by Blaney et al., 1985) and our experiments with **S. littoralis** (in press) show that peripheral responsiveness to a range of compounds is influenced by the diet on which the larvae are reared (see also Schoonhoven et al., this volume). Further, peripheral responsiveness varies through the instar, being high in mid-instar, and varies also with time of day.

It may be that all, or most of these manifestations of variation in responsiveness, both sensory and behavioural, have a common mechanism and that they act in concert with other physiological changes, such as mixed function oxidase activity, to maximise the effectiveness of the insect in a changing environment.

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LEARNING WITHOUT NEUROSIS IN HOST FINDING AND OVIPOSITION BY THE CABBAGE BUTTERFLY, PIERIS RAPAE

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Key Words: **Pieris rapae**, host finding, oviposition, learning, sinigrin, chlorogenic acid, oviposition deterrents, neurosis.

1. Introduction

The more the behaviour of an insect is observed, the more flexible it appears by virtue of different categories of learning. This is exemplified by the host-finding behaviour of the gravid female **Pieris rapae.** The adult female matures several hundred eggs if nectar is drunk and deposits them singly in alternation with flight. Near Canberra, each gravid butterfly traverses many kilometres (Jones et al., 1980). The larva, also, may wander and locate a new host (Jones, 1977) but more decisions about host acceptability are made by the adult female in the course of its thousands of landings on plants (Davies & Gilbert, 1985).

The first report of learning in host finding and oviposition by **P. rapae** concerned an increased responsiveness to green and yellow objects following contact with a cabbage leaf as long as 72 h previously. In the same experiments, the rate of landings on non-host foliage declined during a 30-min test (Traynier, 1979). In addition, butterflies in dual choice experiments learnt to associate the appearance of hosts with their chemical acceptability for oviposition. Data were gathered from observations of butterflies made recognizable by numbered wings and held in cages, through experiments with intact plants, leaf discs and paper discs with sinigrin solution (Traynier, 1984, 1986). Colour vision, as distinct from spectral sensitivity, is unproven in **P. rapae** and colour is used only as a descriptive term in this report. From preliminary experiments of 24 h duration, it was concluded that butterflies did not associate shape or size with the presence of sinigrin solution.

2. New evidence for associative learning

Experiments were continued with discs of different colours using the methods reported previously. 'Shoalhaven' green and white discs against a background of pink curtains were used in recent experiments because butterflies offered a choice were equally likely to land on green or white as an initial choice. These discs were used to provide additional examples of associative learning. Numbered butterflies were placed singly on either a green or a white disc where they made tarsal contact with 200 ppm sinigrin solution, without oviposition, and an hour later were offered a choice of both kinds of disc alternated round a circle. Of 46 butterflies

placed initially in a green disc, 44 landed in the choice test and 36 of these landed on a green disc. Of 46 butterflies placed initially on a white disc, 39 landed in the test and 33 of these landed first on white. The choices by experienced butterflies differed significantly from random ($P<0.001$, 2x2 X^2).

3. Deterrents evaluated by a conditioning technique

As butterflies were known to learn to oviposit on water discs of the same appearance as sinigrin-solution discs present in the same cage, an oviposition deterrent in aqueous solution was tested by offering an additional disc of the same appearance with the candidate material. When fewer eggs were laid on the test discs than on water discs, the test material had deterred oviposition. This method avoided the mixture of oviposition releaser and deterrent which may involve chemical reaction. Moreover, it reduced the likelihood that the butterflies learnt to associate deterrent and releaser and thereafter oviposited in response to deterrent alone, just as blowflies had been conditioned to respond to a usually deterrent solution as if it were a feeding stimulant (Nelson, 1971). A limitation of the method however, was that the test material must not colour the discs. Water must be present also, but may be applied in a non-polar solvent and water added later. When filtered cabbage juice was tested by this method it deterred oviposition, consistent with the results of Renwick & Radke (1985), but while the method seemed promising there was a darkening of the deterrent discs which made the result uninterpretable. Similar darkening occurred with extracts of potato tubers. The following pure substances with colourless solutions were tested as deterrents by this conditioning method: 3 M and 5 M sodium chloride, 1000 ppm chlorogeneic acid (pH 2.6), a saturated solution of quinine sulphate, and 0.1 M citric acid (pH 1.9) and 0.1 M tartaric acid. The sodium chloride and chlorogenic acid solutions deterred oviposition, the others had little effect and were not investigated beyond a preliminary test.

4. Chlorogenic acid as an oviposition deterrent

Six female butterflies were held in a cage through three successive 30-min tests in which six white discs were offered for oviposition, with 90-min intervals between tests. Two of the discs were wetted with sinigrin solution and the remainder were all wetted with water in the first and third tests, while the second test had two discs with water and two with 1000 ppm chlorogenic acid, a polyphenol known from many plant species (Isman & Duffy, 1982). The discs were disposed round a circle. Egg counts (Fig. 1A) showed that most eggs were laid on sinigrin discs, and distributed evenly on pairs of water discs ($P<0.01$, Friedman ANOVA; n = 10 cages). These results were consistent with previous findings (Traynier, 1984, 1986). In the second test, the chlorogenic acid discs acquired almost no eggs although eggs were laid on water and sinigrin discs. The third test yielded an egg distribution with the same pattern as the first. These results implied that chlorogenic acid influenced behaviour only instantaneously as a deterrent and failed to disrupt learning, since oviposition on water discs is known to involve learning.

5. Influence of chlorogenic acid on landing rate

The rates of landing on green discs were compared in a non-choice test between discs wetted either with an aqueous solution of 1% chlorogenic acid or with water alone. Six discs of one kind were offered to five numbered butterflies, in each of a pair of matched cages for simultaneous comparison. To ensure landing the butterflies were placed in tarsal contact with 1000 ppm sinigrin solution on a green disc, one hour before a 20-min period in which landings were recorded. In six repetitions of the experiment, 25 butterflies landed on water discs with a mean of 4.2 ± 0.8 (SE) landings, while 26 butterflies made 4.5 ± 0.8 mean landings on chlorogenic acid discs. This failure of chlorogenic acid to influence the rate of landing is additional evidence of only instantaneous effects. It seems, therefore that oviposition deterrents play only a minor role in the host selection behaviours of **P. rapae** in comparison with the profound influences of sinigrin in the presence of water.

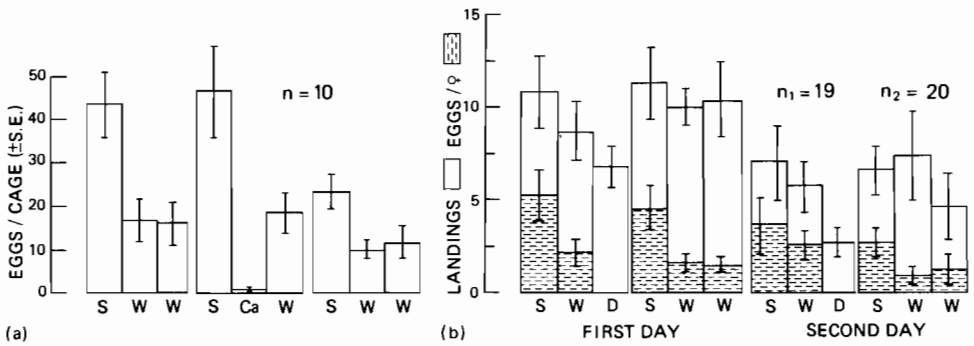

Figure 1. (a) eggs laid on treated discs in three successive tests. S = sinigrin, W = water, Ca = Chlorogenic acid. (b) landings and eggs from single butterflies in paired cages with or without deterrent in two successive tests. D = sodium chloride.

6. Comparison of assay methods

Assays for chlorogenic acid either alone or mixed with sinigrin solution were compared by offering three butterflies a triple choice of the following aqueous solutions on green discs: 200 ppm sinigrin, 1000 ppm chlorogenic acid, and a mixture of both these together. Butterflies were placed on the sinigrin discs to begin a 30-min period of oviposition, from which the mean egg counts (± SE) from ten repetitions were respectively: 27 ± 5, 14 ± 5 and 6 ± 2 ($P<0.001$, Friedman ANOVA). These results indicated that the most sensitive assay offered deterrent and releaser on separate discs.

7. Sodium chloride as a deterrent

Oviposition deterrents may be numerous and have diverse behavioural influences on behaviour. A second material, 5 M sodium chloride, was therefore tested by the conditioning method with concurrent counts of oviposition and landings by numbered butterflies. Two sinigrin-treated

discs, two water discs, and two deterrent discs were placed round a circle and offered in one cage, simultaneously with a cage alongside with two sinigrin discs and four water discs. The deterrent was 5 M sodium chloride solution which required the addition of drops of water during the test to prevent crystals forming by evaporation. Data from 39 butterflies showed no difference in the total number of landings and ovipositions in cages with or without the deterrent, through two 30-min tests with a 24 h interval (Fig. 1B). No continuing effects on behaviour, therefore, were indicated by sodium chloride which resembled chlorogenic acid in this respect.

8. Learning without neurosis

There is thus an accumulation of evidence for a syndrome of learning effects in the interconnected behaviours of host finding and by visual cues and oviposition in P. **rapae**. There was an habituation to non-host materials during repeated landings, a long-term sensitization to yellow and green substrates induced by a tarsal contact with a host, and concurrently an associative learning of the visual and chemical stimuli of hosts. All these effects would facilitate the location of hosts. By contrast, a solution of oviposition deterrent had no more influence on learning than did water. It is well known that mammals and birds become behaviourally disordered (neurotic) when subjected to reward and deterrent in association with a common stimulus. **P. rapae** was immune to neurosis by failing to learn about deterrents and showed no disruption of behaviour other than a failure to oviposit when in tarsal contact with deterrent.

The question of neurosis in insects was raised by Schneirla (1962) who disturbed the behaviour of ants but failed to make them neurotic when he changed the positions of food rewards in a maze which they had learnt previously. In a parallel experiment, rats became neurotic. Schneirla concluded that ants, unlike rats, learnt the maze only in short stages, did not generalize and were insusceptible, therefore, to problem conflict. He interpreted some experiments on disturbed foraging in honey bees along similar lines. The present experiments with **P. rapae** provide a clear example of the inability of an insect to become neurotic.

Some other species of insects do learn to respond to oviposition deterring pheromone, but the stimuli they associate, if any, have yet to be identified. Their mode of learning cannot, therefore, be discussed in this context (van Lenteren & Bakker, 1975; Klomp et al., 1980; Roitberg & Prokopy, 1981; Ikawa & Suzuki, 1982). Phytophagous insects may habituate to feeding deterrents (Schoonhoven & Jermy, 1977; Jermy et al., 1982) and show food-aversion learning (Dethier, 1980; Blaney & Simmonds, 1985) but neurosis was not reported in these studies.

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Labeyrie, V., Fabres, G., Lachaise, D. (eds) Insects – Plants

INFLUENCE OF ALLELOCHEMICAL SUBSTANCES OF THE HOST PLANT (ALLIUM PORRUM) ON DEVELOPMENT AND MOULTING OF ACROLEPIOPSIS ASSECTELLA (LEPIDOPTERA). THEIR ROLE AS SELECTIVE FACTORS

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1. Introduction

Since the discovery of the "raison d'être" of secondary plant substances (Fraenkel, 1959), many examples of insect-plant relationships have been examined with reference to plant allelochemicals. Theories on generalized and specialized consumers, related to the kind of substances elaborated by the potential host plants, are very attractive. However they often are difficult to apply to a great variety of systems, because each system has its own particularities. We propose to examine one example of such relationships, in an agrosystem where the insect is a pest, the leek moth, and the plant a vegetable, the leek.

The leek, **Allium porrum**, is cultivated on large areas in Western Europe. Like the other plants of this genus, it contains very specific sulphuric compounds, responsible of its particular odour and pharmacological properties. **Allium** plants have also specific kinds of stable substances, steroïdic saponins. These plants are attacked by only few insect species: the leek moth, **Acrolepiopsis assectella**, is the most specialized of the consumers of the leek, and it is the only Lepidoptera usually found on this plant. This insect strictly depends on the leek, for development and reproduction; it is able to damage the crops sufficiently to cause economical problems, and has been studied for this agronomical interest.

Allelochemical substances of **A. porrum** have an important influence on the physiology, development and behaviour of the leek moth. Some substances stimulate the insect growth and induce egg-laying, others inhibit the larval development in feeding assays. The first group of substances ("kairomones") is constituted by volatile sulphuric compounds and by stable substances (Auger & Thibout, 1981, 1983). These compounds attract the adult males and females, and induce egg-laying. They allow phagostimulation of new-hatched larvae and continuation of feeding. The presence of the leek is necessary for the best reproductive efficiency and the greatest polymorphism in the populations of the leek moth. In laboratory insect strains, we showed that the host plant and its substances exert selective

pressures on physiology and behaviour of **A. assectella** (Arnault & Loevenbruck, 1986a and b). The volatile substances can also be repulsive for other species of insects (Lécuyer, 1975). The second group of substances ("allomones") acts on **A. assectella** as toxic factor, at least on a part of the populations. These substances have been identified as saponins (Harmatha et al., in press). We present below the results on their biological action and physiological consequences on individuals and laboratory population of the leek moth; we also compare this action with the effect of other substances having similar or antagonistic influence on this insect; finally we suggest hypotheses on the significance of such allelochemicals substances in the plant tissues.

2. Results

2.1. The saponins of **Allium porrum**, their action on **A. assectella**

2.1.1. Description. Steroïdic subtances of **Allium**, specially saponins, have been studied in various species, wild or cultivated (Ismailov & Tagiev, 1980). These substances are more concentrated in the flowers than in the vegetative parts of the same plant; this phenomenon can be related to the floral differentiation. Evidence has been made of a toxic effect on **A. assectella** of leek flower powder added to the diet to replace the leek leaf powder usually added for the breeding of larvae (adults don't feed); this toxic effect was described as high mortality (Arnault, 1975) and inhibition of moulting of young larvae (Arnault, 1979).

From the cultivated leek "Malabare", active water-methanolic extracts were prepared and tested in the diet; we progressively conclude to a steroïdic structure of the active compound, which was finally identified (Harmatha et al., in press); it is identical to the "aginosid" already described in **Allium giganteum** (Kel'Ginbaev et al., 1976). The aginosid concentrations are (dried weight) 0.2 - 0.4% in the flowers and 0.03% in the leaves of **A. porrum**; it has been also found in a "wild leek", **A. polyanthum** (0.02% in the flowers and 0.004% in the leaves). The concentration is thus about 15 times higher in the flowers than in the leaves of a same plant.

The structure of the molecule of aginosid shows great similarities with another saponin more common: the digitonin. We showed that these two saponins cause the same toxic effect on **A. assectella** larval development. This similarity was exploited to study the physiological action of these compounds and their interactions with other sterols.

2.1.2. Action on development and physiology of the leek moth. When leek flowers or aginosid or digitonin are added to the standard diet, they act as ecdysis inhibitors for numerous individuals from laboratory populations of the leek moth. The standard diet is semi-synthetic; it contains maïze semolina, wheat germ, dried yeast, ascorbic acid, benzoïc acid and nipagin and agar-agar. The concentrations of products responsible for 50% mortality are: 20 mg/ml of died flowers, 900 ug/ml or pure aginosid, or 600 ug/ml of digitonin.

A dose-response curve has been established with the digitonin. it was used as a standard to evaluate the equivalent concentration of toxic

saponins, knowing the % of insect mortality. The aginosid seems to be less active than the whole flowers, for equivalent concentrations: this means that it is probably not the only saponin (or the only toxic compound) present in the leek flowers. In our laboratory populations of **A. assectella**, at the concentrations found in the flowers by chemical methods (0.2 - 0.4% of dried weight), the aginosid kills about 50% of the larvae, at the 2nd or 3rd moult. Before dying, the larvae generally have two head capsules (Figure) and two cuticles (the external one irregularly melanized). The gut and the Malpighian tubules contain abnormal concretions. The duration of the larval instar is longer than normal, when the moult does not succeed. We have diagnosed an inhibition of moult, consecutive to an alimentary intoxication.

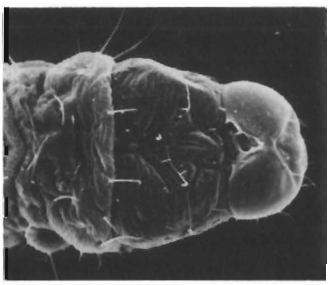

Figure. Electron-micrograph (scanning) of **A. assectella** larva killed after feeding with toxic substances of the leek in the diet: the anterior part with two head capsules and incompletely outcasted exuvia.

The larvae which survive after the 3rd instar are saved, even on saponin-containing diet. Toxicity does not appear if the toxic compound is given only during the first and the beginning of the second instar, before transfering the larvae to a new standard diet. Survivors always achieve their development and the adults are able to reproduce as on the normal food. It is clear that the larvae have a critical stage of sensitivity, before the second moult, as other insects (Coulon, 1977; Slama & Nemec, 1981).

2.1.3. Antagonism with other sterols. Cholesterol is known to form complexes with digitonin. In **A. assectella** we observed an antagonistic effect between these two substances in the larval diet (Arnault & Mauchamp, 1985). The complex is probably elaborated in the larvae, from their own cholesterol taken during the feeding period. β-sitosterol, more common in plants, can also antagonize digitonin but is less efficient than cholesterol; the β-sitosterol taken from food must probably be first converted into cholesterol by the larval metabolism. Antagonistic effect exists also between cholesterol (or β-sitosterol) and dried leek flowers, methanolic extract, and aginosid. Such effect appears to represent an efficient way of detoxication: in the young larvae by taking cholesterol in the food, and in the old larvae by using metabolized cholesterol. This detoxication system is not specific, however, against a particular molecule among the family of the saponins.

2.2. Action of hormone-analogs on **A.assectella**, compared with the action of saponins

By oral injections of phytoecdysones, Kubo & Klocke (1983) have obtained morphological symptoms of ecdysis disturbances in several species of insects. The description of these abnormalities suggested that the intoxication of **A. assectella** by saponins could be also the consequence of an hormonal unbalance. We looked for the effects of phytoecdysones when added to the diet of the leek moth (Arnault & Slama, in press). The symptoms were at first sight similar than those obtained with saponins, but some differences existed:

- the phytoecdysones act on all the moults, including pupation and emergence; the saponins inhibit only the 2nd and 3rd moults, whatever the concentration is (in the toxic range);
- the hormone-analogs are not apparently responsible of digestive troubles: after ingestion, the dead larvae remain transparent, and nothing appears in Malpighian tubules;
- with phytoecdysones, abnormal moults occur sooner than usual; it is the opposite with saponins;
- no change of the toxic effect is observed when cholesterol (or β-sitosterol) is added to the diet simultaneously with phytoecdysone. The mortality and the symptoms remain identical (see the table). This agrees with the fact that these compounds are not able to form complexes with ecdysteroïds.

Table. Larval mortality of **Acrolepiopsis assectella** in semi-synthetic diets with: 20-OH ecdsysone (20-OH E), or/and digitonin (D), or/and chloresterol (CH).

Substances	concentration µg/ml	larvae nb	mortality %	symptoms %
no	-	315	28 ± 5 (a)	0
20 OHE	100	113	69 ± 8 (c)	16
D	600	360	59 ± 5 (b)	6
20 OH E + D	100 + 600	248	96 ± 2 (d)	38
no	-	223	13 ± 4 (a)	0
20 OH E	100	234	91 ± 4 (b)	9
20 OH E + CH	100 + 400	214	86 ± 5 (b)	11
20 OH E + CH	100 + 800	210	79 ± 5 (b)	20
D	600	373	62 ± 5 (c)	14
D + CH	600 + 400	443	44 ± 5 (b)	2
D + CH	600 + 800	381	27 ± 4 (a)	0

Values with the same letter are not statistically different ($P < 0.001$, Student test).

If phytoecdysones are given together with saponins, we observe an additive effect for the characteristics of mortality and symptoms. The two kinds of compounds seem to act independently. We believe that the toxicity of the saponins is not directly hormone-related: indeed the inhibitin of ecdysis can be a consequence of various factors acting during development.

2.3. Location of the saponins of **Allium porrum** flowers. Behaviour of **A. assectella** on these flowers

In plants, saponins are mostly located in peripheric tissues, as teguments of seeds; they exist in chlorophasts (Murakami et al., 1983). With flowers of leek, mortality and symptoms appear when the larvae feed on the corolla and on the fruit-walls (fresh of mixed to the diet). We have observed that, on the living host plants, the larvae avoid to feed on these parts; they only pierce a hole on them to penetrate the fruit after hatching, then feed on the internal part, and go out at the 3rd instar; they finish their development in the receptable of the inflorescence.

The behaviour of the larvae on the flowers is one fact, the presence of the saponins in high concentrations in the avoided parts is another fact, and it is perhaps difficult to establish a strict correlation, but it is legitimate to ask if the physiological action of saponins on phytophagous insects can be the result of a plant defense system.

2.4. Toxicity of the flowers of other **Allium** on the leek moth

Five cultivars of **A. porrum** were tested: their flowers were all toxic but with an important variability in the % of larval mortality. Flowers of onion (**A. cepa**) and chives (**A. schoenoprasum**) were not toxic for the leek moth. The fact that these plants are cultivated does not help to make hypothesis on a natural coevolution. We indeed found toxicity in the flowers of a wild garlic (**A. rotundum**) and in a wild leef(**A.polyanthum**) that contains aginosid. This latter leek does not attract adults of the leek moth in experimental conditions (Lecomte & Thibout, 1984), but larvae of the leek moth are perfectly able to develop on it in the laboratory, as well as on the cultivated leek. In nature it exists an asynchrony between the life cycles of the plant and of the insect: in this case the natural equilibrium completely eliminates the predator from the potential host plant.

3. Discussion

A. porrum shows very efficient "strategies" to survive after insect damages (Boscher, 1979). On vegetative and on reproductive phases of the plants, the action of the insects can induce faster growth and vegetative reproduction: the consequences for natural selection are not the same as the situation without predators, for the populations of plants. Another good strategy is to avoid damages in parts of the plants which cannot regenerate. In this way we have seen that the flowers seem "protected" by allelochemical substances such as saponins. This is also observed with the tegument of legume's seeds (Janzen et al., 1977). The presence of substances reducing the digestibility of tissues in certain parts of the plants can also regulate the populations of the insects, and preserve a

part of the sexual reproductive potential of the plants. In the case of **A. assectella**, a part of the population is able to detoxify the substances of the plant defense system, sufficiently to maintain an equilibrium between the producer and the consumer. The actual relationships between **A. assectella** and **A. porrum** in the agrosystems are very tight if we consider the overall populations. The leek moth could be controlled by making stronger the natural defenses of the plants, for example by breeding varieties of leek with much higher saponin content. But in the insect populations the individual variability is great. Some individuals do not depend on the host plant for one of several sequences of their development and reproduction; they are eventually able to find new food sources, and to react to new selection pressures.

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SUGAR ALCOHOLS AND HOST PLANT SELECTION IN YPONOMEUTA (LEPIDOPTERA/YPONOMEUTIDAE)

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1. Introduction

Small ermine moths of the genus **Yponomeuta** Latreille are mainly found on host plants belonging to the Celastraceae or the Rosaceae. The Celastraceae are thought to be the ancestral host family (Gerrits-Heubroek et al., 1978). In the literature the sugar alcohols dulcitol and sorbitol, respectively, are recorded as characteristic for the two families mentioned above (Hegnauer, 1964, 1973). Therefore, in van Drongelen's (1980) study of the specificity of the larval gustatory sense organs in **Yponomeuta**, special attention was given to these compounds. He demonstrated a good correspondence between host plant specificity and larval taste response. However, one notable exception was found: **Y. evonymellus** feeding on **Prunus padus** (Rosaceae), the neurons in both the lateral and medial styloconic sensilla were stimulated by both sugar alcohols instead of by sorbitol only. In behavioural tests (Gerrits-Heybroek et al., 1978) it was found that **Y. evonymellus**, when given the choice between leaf discs of five foodplants of small ermine moths, preferred its own foodplant, but a clear second choice was for **Euonymus europaeus** (Celastraceae). A well marked third choice was for **Crataegus** (Rosaceae). None of the other four species tested showed a comparable distribution of choices. **Y. cagnagellus**, sensitive only to dulcitol, did not accept leaf discs other than of its own foodplant **Euonymus europaeus**. In this paper new data on the chemical analysis of the leaves of some Celastraceae and Rosaceae is presented, along with results of experiments with larvae of **Y. evonymellus** and **Y. cagnagellus**.

2. Procedures

2.1. Chemical analysis

Sugar alcohols were determined by gas chromatography after treatment of aliquots of plant extracts with n-butylboronic acid (Eisenberg Jr, 1971; Wallaart, 1980). The leaves were taken from plants in the field on which small ermine moth larvae were feeding.

2.2. Feeding experiments

A twig with four or five leaves of **Prunus padus** was placed in a 1% dulcitol solution in water and left for a) a varying number of hours (as in Figure 1) or b) for 24 hours (see Table 2). In a third test "neutral" leaf discs obtained in the manner described by van Drongelen (1980) were used (see Table 3). Larvae were collected in the field in their second or third

instar. Wen necessary they were reared in the laboratory till the required instar. Rearing and the experiments were performed in a climatic chamber at T 20°; L: D - cycle 17:7.

As 37% of the larvae appeared be parasitized by **Ageniaspis fuscicollis** not all of them reached the pupal stage. In the first experiment of table 2 36 larvae were killed by the parasites at the end of their last instar. Due to mortality at an earlier stage the number of parasites could not be established in the two other tests of Table 2.

3. Results

The dulcitol and/or sorbitol content of the leaves of one species of Celastraceae and of five species of the Rosaceae is given in Table 1. To our surprise two sugar alcohols were detected in the leaf extract of **P. padus:** sorbitol and a small amount of dulcitol. In the near future a more detailed account of these analysis will be published by one of us.

Table 1. Sugar alcohol content (in %dry weight) of leaves of six plant species. Those marked + were obtained in our institute by R.A.M. Wallaart and Th.P.M. van de Water (unpub.obs.).

Family	Species	Sugar alcohol	
		dulcitol	sorbitol
Celastraceae	*Euonymus europaeus*	4.3	-
Rosaceae	*Prunus padus*	0.2	4.0
	Prunus padus		5.8+
	Prunus spinosa		8.0+
	Sorbus aucuparia		8.5+
	Crataegus monogyna		12.6+
	Amelanchier sp.		12.7+
	Amelanchier sp.		13.6+

The small amount of dulcitol in the leaves of **P. padus** is not enough to evoke a feeding response in the larvae of **Y. cagnagellus** (Table 2). With increasing impregnation time the amount of dulcitol increases. It was by checking this assumption that the discovery of dulcitol in **P. padus** was made! In figure 1 it is demonstrated that the feeding response increases correspondingly.

The data in Table 2 also make clear that dulcitol is not the only factor that determines the suitability of **P. padus** for **Y. cagnagellus.** The survival and pupal weight remain far below normal values. As a rule developmental time also increases as suitability decreases. In this case, however, there is no difference in the developmental time of larvae reared on **Euonymus** and larvae reared on **P. padus** plus dulcitol.

Table 2. Performance of L2/L3 larvae of **Y. cagnagellus** when fed with leaves of its own foodplant or with **P. padus** leaves impregnated with dulcitol.

Plant	N =	Number of pupae	moths	Pupal weight in mg	Duration larval period in days
Euonymus	100	61[+]	61	37.5 ± 5.8	19.5 ± 0.9
P.padus + dulcitol	100	38	28	11.4 ± 3.7	20.1 ± 1.5
P.padus	100	0	0		

Finally, the preference of **Y. evonymellus** for dulcitol and sorbitol was measured as is shown in Table 3. Surprisingly the preferred sugar alcohol for this small ermine moth appeared to be dulcitol, although sorbitol is the dominant sugar alcohol of its host plant **P. padus** (Table 1).

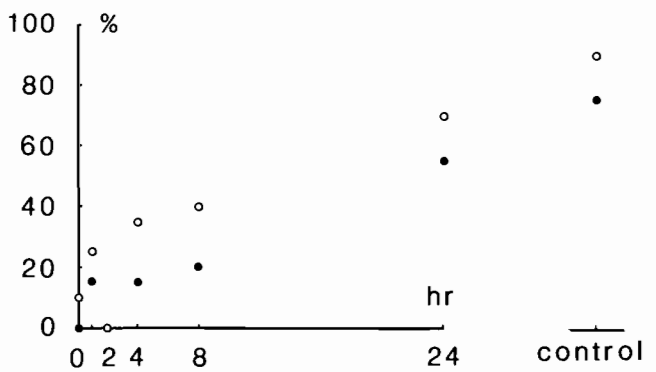

Figure 1. Feeding response of 5 x 20 larvae of **Y. cagnagellus** towards leaves of **P. padus** impregnated with dulcitol during 0, 2, 4, 8 and 24 hrs compared with the reaction to **Euonymus** leaves (control). Dots refer to number feeding after 2 days, open figures after 4 days.

4. Conclusion and discussion

The enigmatic sensitivity of larvae of **Y. evonymellus** for dulcitol has a logical explanation, if it can be proven that they do indeed perceive the low concentration of dulcitol found in the leaves of **P. padus** (Table 1). The preference for dulcitol above sorbitol (Table 3) might be an indication in that direction.

Table 3. Preference of larvae of **Y. evonymellus** when given the choice between "neutral" leaf discs impregnated with sorbitol (S) or dulcitol (D). In each petridish two larvae were present at a time.

Larval instar	Number of tests	Choice situation	Result	No choice
L4	20	1% S vs 1% D	1 : 12	7
L5	20	1% S vs 1% D	2 : 14	4
L4	20	8% S vs 1% D	6 : 10	4
L5	20	8% S vs 1% D	4 : 12	4

For **Y. cagnagellus** much higher concentrations of dulcitol in the leaves of **P. padus** are needed to overcome their unpalatability (Figure 1). As is demonstrated in Table 2 other factors determine the suitability of non-host leaves as well. Several of them are presently being studied and will be discussed in forthcoming papers (Fung, in prep).

The fact that larval developmental time, however, does not differ from that on the normal host might indicate that a positive change of the palatability of this unfamiliar host has been achieved. The importance of sugar alcohols as gustatory stimuli in the feeding behaviour of small ermine moths is also receiving further attention in our laboratory.

The detection of a foodplant of an **Yponomeuta** species containing at the same time the two sugar alcohols playing a paramount role in the feeding behaviour of representatives of this genus, might offer a key for understanding host plant shifts during evolution.

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HOST PLANT RELATIONSHIPS AND SPECIATION IN LEAF-MINING AGROMYZID FLIES ON UMBELLIFERAE

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1. Introduction

Twenty years ago, Bush proposed how two distinctive features of the radiation of phytophagous insects - great species diversity and host specificity - could be causally related, in his model of speciation via formation of host plant races. Working on **Rhagoletis** (Tephritidae), he proposed a model of sympatric speciation with the following important elements: (1) host specificity; (2) mating occurring on the host plant , both sexes being attracted to the host; (3) divergence of populations associated with different hosts (a) initiated by assortative mating among individuals genetically predisposed to select a particular host, and (b) reinforced by directional selection, in which each host-associated population becomes increasingly adapted to its host, leading, for example, to allochronic isolation by synchronization with the host or other ecological factors.

As Bush has emphasized, however, the mode of speciation prevalent in a particular animal group largely depends on features of the biology of the group in question. Therefore, the biological diversity of phytophagous insects may be expected to lead to diversity in the processes of speciation. Zwölfer and Bush (1984) generalized this model to other groups of phytophagous insects and presented a framework for an analysis of speciation.

Leaf-mining Agromyzidae (Diptera) share many features of the biology of **Rhagoletis** that are implicated in speciation via host-race formation. We have initiated an analysis of speciation in this group of phytophagous insects on umbelliferous plants to investigate how applicable Bush's model may be to speciation in other groups of phytophagous insects.

2. Biology of leaf-mining agromyzidae

Leaf-mining agromyzids of the genus **Phytomyza** are specious, highly host-specific endophytophagous flies. Closely related species often occur on different host plants, indicating that, as in **Rhagoletis**, speciation often is associated with a host plant shift. Mating occurs on the host. The different host plants differ strikingly in phytochemistry and ecology, which may lead to selection for divergence. Also as in **Rhagoletis**, **Phytomyza** spp. have a highly aggregated niche structure (Zwölfer & Bush, 1984). Most activities are tightly associated with the host, so that host plant choice strongly influences adult and larval habitat and food, as well as mating biology, pupation and hibernation sites.

Adults emerge in the spring after overwintering as pupae in the soil near the host. Adults of both sexes are attracted to the host plant. Females use their boring ovipositors to make large numbers of "feeding punctures" in the leaves of their host, and eat the cell sap that exudes. On this diet, these tiny flies can live for a month or more. Males come to the host plant to mate. Eggs are laid in leaves of the host. Development from egg to pupa takes about 9-12 days. The fully-grown larva cuts an exit slit and drops to the ground, pupating in the upper layers of the soil.

Leaf-mining **Phytomyza** spp., however, differ from **Rhagoletis** in ways that may be important in processes of speciation. First, in contrast to frugivorous **Rhagoletis**, leaf-mining **Phytomyza** feed on well-defended plant parts, characterized by strongly divergent secondary chemistry. This suggests that survival genes may be more important here than in **Rhagoletis** feeding on ripe pulpy fruits, which are among the least defended parts of plants. Second, leaf-mining **Phytomyza** feed on mature leaves available throughout the growing season, allowing more than one generation per year. This suggests less opportunity for allochronic isolation than in the largely univoltine **Rhagoletis** spp. associated with a seasonally more restricted food source.

3. Materials and methods

3.1. Systems studied

Among the **Phytomyza** spp. associated with umbelliferous hosts in Central Europe, three systems common in our region and differing, according to the literature, in degree of divergence, were studied in detail:

(1) **Phytomyza spondylii** - a species recognized in the most recent literature (Griffiths, 1973) as two distinct species, **P. spondylii** and **P. pastinacae**, each feeding on both **Heracleum sphondylium** and **Pastinaca sativa;**

(2) **Phytomyza chaerophylli** - an oligophagous species feeding on different hosts of the tribe Scandiceae; its principal hosts in Central Europe are **Anthriscus silvestris** and **Chaerophyllum temulum.** Nowakowski (1962) suggested it may be a complex of monophagous species of host races; and

(3) **Phytomyza angelicae** - feeding on **Angelica silvestris,** and recently lumped by Griffiths (1973) with **P. laserpitii** on **Laserpitium latifolium.**

3.2. Field studies

Detailed field ecological studies, including population dynamics of the umbelliferous hosts as well as of the leaf-mining flies, were carried out during 1983-1986 in about 30 populations in the southwestern parts of the Swiss Jura and the Upper Rhine Valley. Populations were sampled regularly to provide material for the establishment of laboratory stocks, and for population-genetical investigations using enzyme electrophoresis.

3.3. Laboratory studies

Laboratory studies included experiments on host plant choice, host suitability and mating behaviour, as well as phytochemical investigations (TLC) of the umbelliferous hosts and population-genetic studies of the insects using horizontal starch gel electrophoresis methods described by

Menken (1982). A total of up to 18 enzyme systems were investigated and analysed using Wright's (1951) F-statistics.

4. Results and discussion

4.1. Population stucture

Our analysis of population structure, focusing on F(st), shows that theses flies occur in populations with a high degree of local genetic differentiation. F(st) is a measure of the degree of differentiation among local subdivisions of a population of a species - in this case, among local populations of the total species population. If F(st) is zero, then no local differentiation exists. Some very mobile butterflies like **Danaus plexippus**, **Pieris rapae**, and **Yponomeuta cagnagellus** approach this situation (Table 1). In contrast, in the **Phytomyza** species investigated, high F(st) values are found, comparable to those of sedentary species like **Euphydryas editha**, known to exist in highly local and well differentiated subpopulations. Populations of these leaf-mining flies thus possess the kind of population structure that has been postulated to lead to host-associated divergence in **Rhagoletis**. Further F-statistics, F(is) and F(it), as well as results from our field studies, also indicate that local populations of these agromyzids are small and highly inbred (Table 2).

Table 1. Comparison of the standardized variation (Fst) in different insect taxa (See Latscha, 1986 for references).

Species	Family	F(st)
Danaus plexippus	Nymphalidae	.009
Pieris rapae	Pieridae	.014
Yponomeuta cagnagellus	Yponomeutidae	.027
Drosophila pseudoobscura	Drosophilidae	.030
Drosophila melanogaster	"	.044
Drosophila robusta	"	.055
Phytomyza laserpitii	Agromyzidae	.088
Euphydryas chalcedona	Nymphalidae	.090
Euphydryas editha	"	.118
Phytomyza chaerophylli	Agromyzidae	.120
Phytomyza angelicae	"	.148

In summary our results indicate that population structure is such as to allow local divergence of populations, including divergence associated with different host plants.

Table 2. F-statistics (Wright, 1951) for three **Phytomyza** species.

Species	F(is)	F(it)	F(st)
P. chaerophylli	.378	.442	.120
P. angelicae	.433	.513	.148
P. laserpitii	.779	.800	.088

4.2. Host-associated divergence

Is there selection that would drive such divergence? In two of the three cases studied so far, at best very slight divergence caused by genetic drift in small, isolated populations and/or habitat-specific selection could be recognized among populations, suggesting that in these cases such mechanisms are unlikely to produce new species.

(1) **P. spondylii** turned out to be a single species, oligophagous on **H. sphondylium** and **P. sativa**. All females accept both hosts for oviposition and feeding, and no genetic differentiation could be detected so far, resulting in an overall genetic identity (I) between flies on different hosts of I = .895 (Saner, 1986) comparable with values found for conspecific populations on other insect taxa.

(2) Similar results were obtained for the oligophagous **P. chaerophylli** on **A. silvestris** and **C. temulum**. Again, all females accept both hosts for oviposition and feeding, and almost no genetic divergence could be found (overall genetic identity I = .974). However, slight but significant differentiation was detected at one locus (Pgm), in which the same alleles occurred at different frequencies in populations associated with different hosts in allopatric situations; where both hosts occurred sympatrically, electromorph frequencies were intermediate (Frey, 1986).

Our so far limited data on host plant secondary chemistry come from TLC of polyacetylenes, coumarins and terpenoids of leaf surface waxes. Though there are quantitative differences of composition, the hosts of **P. spondylii** (**Heracleum** and **Pastinaca**) and of **P. chaerophylli** (**Anthriscus** and **Chaerophyllum**) contain qualitatively similar mixtures of these compounds. Also, the alternative hosts of each of these two leaf-miners grow in very similar habitats and often occur together. Furthermore, the hosts of each species are characterized by nearly identical vegetative phenologies, and - both fly species having multiple overlapping generations - leave little opportunity for allochronic isolation (Fig. 1).

(3) Results for "**P. angelicae**", however, strongly indicate that host-associated divergence and speciation have occurred (Latscha, 1986). "**P. angelicae**" was revealed to consist of two closely related sibling species, **P. angelicae** monophagous on **A. silvestris** and **P. laserpitii** monophagous on **L. latifolium**, differing in four completely diagnostic loci (Idh, Est -1, Est -2, Lap). This indicates that there is effectively no gene flow between them, even where they occur sympatrically. Nei's genetic identity for the two species is I = .591, a value comparable to sibling species in other insect genera, e.g., **Ectoedemia** (I = .547; Wilkinson et al., 1983), **Drosophila** (I = .563; Ayala et al., 1974), and **Rhagoletis** (I = .741; Morgante et al., 1980).

The host plants of these two very closely related **Phytomyza** spp. are much more divergent chemically and ecologically than in cases (1) and (2). While a similar pattern for polyacetylenes was found in both species, **Angelica** gaves spots that match with linear and angular furanocoumarins, whereas in **Laserpitium** - at least in leaves of this one species - no furanocoumarins could be detected. Terpenoid patterns of the two plants are also quite different. Furthermore, the hosts of this sibling species pair tend to occur at different elevations (although there is broad overlap),

and show marked differences in habitat preference and phenology (Fig. 1).

Transplantation experiments showed that **P. angelicae** larvae can survive in **Laserpitium** but that **P. laserpitii** cannot in **Angelica.** This suggests that speciation in this sibling species pair may have been initiated by a shift form **Angelica** to **Laserpitium** requiring only evolution of the host-recognition mechanism followed by adaptation to the habitat and phenology of **Laserpitium** (Latscha, 1986).

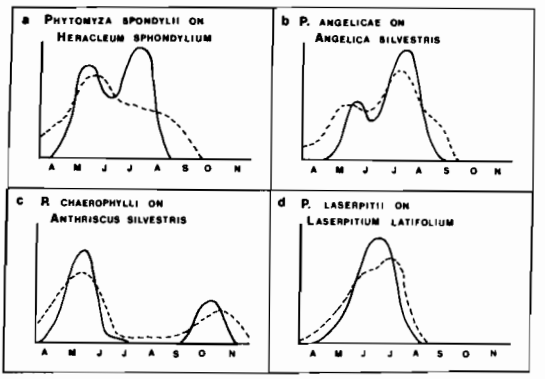

Figure 1. Phenology of host plants (---) and flies (——).

5. Conclusion

Our field and laboratory studies indicate that divergence in these flies is strongly influenced by characteristics of their biology, and that processes of speciation may be different among closely related agromyzids. In oligophagous species, like **P. chaerophylli** and **P. spondylii**, speciation in allopatry is most probable, while **P. angelicae** and **P. laserpitii** may have diverged in sympatry via formation of host plant races. Further studies will be conducted to unravel the mechanisms of speciation and adaptative radiation in these leaf-mining Agromyzidae.

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CHAPTER 6. SELECTION OF CULTIVARS, POLYMORPHISM OF INSECTS AND RESISTANCE OF PLANTS

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INTRAPLANT GENETIC VARIABILITY (TOPIC OF A ROUND TABLE DISCUSSION)

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The hypothesis that individual aborescent plants are mosaics with genetic variation has been revived recently by Whitham & Slobodchikoff (1981). Whitham et al., (1984), and Gill (1986) review nearly 500 published papers and confirm that the hypothesis is consistent with the established principles of plant genetics, plant organogenesis and architectural development, the patterns of pest-plant ecology and evolution, and modern practices of propagating new woody cultivars. This communication reviews only the main features of the hypothesis; the reader is referred to the papers above for documentary literature.

Developmental-Origin and Fate of Intraplant Genetic Mosaicism

As trees and cloning grasses grow, the number of branches increases sigmoidally from the single germinating shoot to a maximum of 10^4 - 10^6 (depending on species) shoot tips at maturity. Repetitious in morphogenetic structure, the branch modules gain developmental and topophytic independance. Thus, a large tree is really a metapopulation of modules with distinct age structure and demographic dynamics, with each module undergoing a complete life cycle of birth, growth, maturation, senescence, and death (White, 1979).

It is postulated that genetic diversity among the parts of highly branched plants is generated by the accumulation of developmental mutations that arise spontaneously in the meristems of the proliferating branches. A diverse array of mutations (point, transposonal, cytogenetic, plastidic, etc.) are known to occur within individual meristems. Klekowski & Kazarina-Fukshansky (1984a, b) have shown in computer simulations that mosaic shoots do emerge when neutral mutations occur in one or several germinative cells in a meristem. If the mutation is deleterious, its rate of loss accelerates as both the number of initials and the number of cell division between selections increase. Conversely, if the mutation is beneficial, it is expected to fix in the shoot. Thus, an arborescent plant can become increasingly complex genetically as it grows and expands.

Many but not all mutations that occur spontaneously in cells of shoot meristems will show gametophytic inheritance. Although the progenitive cells (initials) of plant apical meristems are totipotent, specific plant tissues do differentiate from distinct layers in the meristem. While the long historical controversy over the histogenesis of vegetative and floral structures is not yet settled (Stewart, 1978; Klekowski & Kazarinova-Fukshansky, 1984a), most dicots seem to have three primary layers and

pollen and ovules seem to derive from the L2 layer. Thus, only those mutations that occur in the L2 layer have the dual potentiality of expression in both vegetative tissues (e.g. mesophyll) and inheritance through the gametophytes. Spontaneous mutations that occur in the initials of the L1 (which produces epidermis) and L3 layers (which generates the deep vascular system) are normally not transmitted through seed.

These observations recommend an important clarification in the usage of the term "plant somatic mutation". Because the separation of germ and somatic cell lineages (Weissman's Rule) is fundamental in animal development (Buss, 1983), the term "somatic mutations" has precise meaning in zoology. In contrast, the double potentiality of L2 mutations in plant meristems requires that they be called "developmental" mutations to distinguish them from genuine "somatic" mutations that are restricted to non-gametogenic tissues.

Of course, phenotypic variation among units within clones also arises during development from other sources besides genetic mutation. Microclimate and disease induce position-dependent developmental phenotypes. Meins and Binns (1979) discuss how certain developmental (habituated or genetically differentiated) states can be stable (e.g. terminal and lateral shoots of Norfolk Island pines) and persist in propagation. When used as scions, juvenile and mature shoots can have significantly different courses of growth and development.

Whether developmental mutations in dynamicaly growing plants do in fact have significant influence on evolutionary rates in plants has been considered mathematically by Slatkin (1984), and Antolin and Strobeck (1985). As independent shoots emerge, the extent of genetic mosaicism in the crown of a highly branched plant depends upon the rate of mutation and the relative magnitudes of selection for and against the new mutants. Antolin and Strobeck (1985) concluded that "somatic" mutations can be a potent source of variability in large, long-lived plants, so long as the rates per branch are as high as 10^{-4} or higher. Slatkin (1984) also concluded that "somatic" mutations can have a significant effect on plant evolution but only if the "somatic" mutations rates are much greater than gametic mutation rates and if the selective advantages of invading mutants are substantial.

Evolution of Plant Resistance to Pests

Since the publication of the seminal papers by Fraenkel (1959, 1969) and Ehrlich and Raven (1964) the concept of plant-pest coevolution mediated by secondary plant compounds has enjoyed wide popularity. Many plant species are polymorphic with respect to secondary compounds (Crawley, 1983) and herbivores do discriminate among plants according to their chemical composition. However, it is not understood how exceptionally long-lived plants can evolve new adaptive mechanisms of resistance rapidly enough to remain in perpetual battle with short-lived, fast evolving enemies (Stebbins, 1958; Williams, 1975).

Whitham (1983) has proposed that small-scale, heterogeneous patterns of resistance presented by variable foliage within arborescent plants may effectively impede pests from tracking, by adaptive change, the mechanisms

of plant defense. Meanwhile, as resistant branches grow vigorously and damaged susceptible shoots decline, genetically mosaic trees become increasingly resistant as they grow. Yet, the remolding of the genetic mosaicism is a dynamic process because the species composition of pests changes every year, and different resistant genotypes within the plant will be favored in successive growing seasons. In mature canopy trees, this process may be very important because the steady state number of apical meristems in mature trees implies that twig-shedding and new bud production occur at nearly the same rate.

Gill (1986) emphasizes the additional fact that foliate branches produce larger yields of fruit and seed than defoliated branches (Crawley, 1983, this symposium). As gametophytes carry the same mutant alleles as the mesophyllic layers in resistant leaves adjacent to the flowers, high yields of seed and pollen from genetically resistant branches will amplify the frequency of resistant genotypes in the total progeny of the year. Thus, natural selection among the genetically diverse branches within a tree occurs on a yearly time scale, and evolution of adaptive mechanisms of resistance in large plants can be many times more rapid than that predicted by their otherwise slow demographic generation times. Hence the hypothesis of intraplant genetic mosaicism offers a partial solution to the dilemma of how long-lived plants can co-evolve resistance to short-lived enemies.

Strong support for the view that among-branch variation in resistance plays an important role in the coevolution of plants and their enemies is provided by the stimulating work on Black Pine Scale infestations of Ponderosa Pine (Edmunds & Alstad, 1982), and pemphigine aphids on cottonwood (Whitham, 1983). Through elegant experimental manipulations, both research groups have shown that the fitness of the homopterans strongly responds to the characteristics of individual branches and that a genetic basis to much of the variation in the tree is strongly suspected.

Breeding Systems of Plants

Because seedlings that are genetically different from parent plants may escape localized pathogens (Augspurger & Kelly, 1984), breeding mechanisms that promote genotypic diversity in seeds can have high selective value for individual trees. In the traditional concept of plants as monogenotypic individuals, cross-pollination from separate individuals (xenogamy) is assumed to be the principle mechanism whereby genetic recombination occurs. Mass-flowering in animal-pollinated species with bisexual flowers has puzzled evolutionary botanists because of the high cost in nectar and pollen rewards to pollinators for low yields (1/4 1%) of out-crossing (Augspurger, 1980). Arroyo (1976) suggested that breeding systems entailing excessive geitonogamy (pollinations among flowers on the same plant) rapidly evolve mechanisms of self-incompatibility to avoid the selective disadvantages of inbreeding.

However, geitonogamous pollination within genetically mosaic plants could effectively recombine the genetic variants present within the plant. Thus, mass-flowering by large, self-compatible plants not only promotes xenogamous pollination but also opens a rich store of intraplant recombination. But self-compatilibity is essential for these selective

benefits. In effect, the hypothesis of intraplant genetic mosaicism predicts a polarity in the evolution of plant breeding systems that is diametrically opposed to Arroyo's (1976): the larger the plant, the greater the expected diversity of genotypes among its parts, the greater the benefits accruing from recombining those genotypes, the greater the selective advantages of mass-flowering and self-compatibility.

The reported high frequency of self-compatibility in hermaphroditic species of trees (Bawa et al., 1985) seems to contradict the theoretical advantages of intraplant recombination. However, the standard technique of testing the compatibility system of arborescent plants may artificially inflate the estimates of the frequency of self-imcompatibility (Gill, 1986). For good practical reasons, the tests usually include only autogamy (self-pollinating the same flower) or geitonogamy of immediately adjacent flowers on the same branch; in both cases the genotype of pollen and stigmata are expected to be identical, and therefore incompatible (Mulcahy & Mulcahy, 1983). The standard tests have not looked for genetic variation in self-incompatibility among branches within trees. Cases such as 'Golden Delicious' apples and **Cordia alliodora** (Boraginaceae) in Costa Rica (Bawa, 1974) which exhibit variability in their compatibility systems deserve detailed investigation.

Evidence of Genetic Mosaicism

The literature provides considerable evidence of genetic mosaicism within plants (reviewed on pp. 25-29, Gill, 1985). Among the best known are the cytogenetic variants in spring beauties (Lewis et al., 1971), and the bud sports of ornamental trees, shrubs and flowers (Dermen, 1960). Perhaps most spectacular are the variegated shade trees (**Acer**, **Ilex**, etc.) and the unstable sport azaleas (**Rhododendron** spp.). The cultivars of seedless grapes, Navel oranges and pink grapefruits are all cloned derivatives of spontaneous bud sports. It has been known for over 2000 yr that old grape varieties rapidly become chimeras and degenerate with time; we know that this instability is due to spontaneous mutations (Becker, 1977). Genetic variation among tillers within individual genets of pasture grasses has been revealed by cloning experiments (Breese et al., 1965; Libby & Jund, 1962).

Although foresters advocated the exploration of within-tree genetic variation as a potentially rich source of heritable material for pest resistance in forest trees over 20 years ago, there does not exist any investigation (to my knowledge) that has systematically quantified the genetic variation within large, arborescent plants. There are a few studies of allozymic (electrophoretic) variation in forest trees (mostly conifers, a few woody angiosperms, and some cultivated trees (especially **Citrus**) but they have all been at the population level (Hamrick et al., 1981). However, intraplant genetic variation is found in all of the commercially important fruit trees and is routinely exploited for the selection of improved cultivars. While most of the major varieties of apples were discovered accidentally as single seedlings (Wynne, 1975), nearly all of the important cultivars of apples have been derived from selected bud sports (Zimmerman, 1980 p. 78).

Recent research on somaclonal variation in micropropagated crops (Evans et al., 1984) provides a first test of the theory of genetic mosaicism. Somaclonal variation is now documented in more than 30 crop species, including the cereal grasses, alfalfa, banana, carrot, celery, corn, lettuce, oil palm, and sugar cane (Miller, 1985). Curiously, the richest diversity of somaclonal variants has been recovered from tissue and cell cultures of tomatoes, potatoes and tobacco, all members of the family Solanaceae. The variation includes dominant, semidominant, and recessive gene changes at new and previously mapped loci, large and small chromosomal aberrations (polyploidy, deletions, rearrangements, etc), and cytoplasmic variants (Evans & Sharp, 1983).

Novel mutations are recovered from non-callus cultures at a routine rate 1 out of a 1000, and single gene mutations in callus occur at a frequency of 1 - 5% (Zimmerman et al., 1986). These reports more than satisfy the theoretical requirement (above) that developmental mutation rates be at least 10^{-4} for intraplant genetic mosaicism to have evolutionary importance. The difference in the rates of recovery in the two culture systems could be the consequence of normal cells overgrowing less vigorous mutant cell lines in the differentiated tissues. First viewed as hampering the micropropagation of new cultivars, somaclonal variation is now enthusiastically embraced as a rich source of heritable variation in morphology, yield, and pest resistance (Miller, 1985).

It is widely believed that the procedures of micropropagation are themselves unnaturally mutagenic. The kind and concentration of auxins, cytokinins and other growth regulators used in their culture strongly affect phenotypic and genetic stability (Doorenbos, 1977). Unconscious changes in virus infections may be critical. In fact, most of the ambient conditions of light and temperature, as well as condition of the culture medium must be optimal in order to control the frequency of off-types (Zimmerman et al., 1986). While there is no question that culture conditions are important, there is also no compelling evidence to exclude the possibility that some of the extraordinary amounts of somaclonal variation found in the commercial plant industry were present in genetically mosaic source-plants. In fact, most of the somaclonal variants in tomatoes either occurred very early in the culture process or were present in the original explant because the resultant plants are entirely mutant.

Because of the far-reaching implications of the theory of genetic mosaicism within plants, it is important to determine its prevalence in nature, and distinguish the possibilities of naturally high mutation rates in meristems, cell-line selection, and artifactual mutagenesis. Discovering the interaction between natural mutation rates and natural selection among modular parts would have significant impact on basic theory in population genetics and evolutionary biology, as well as commercial horticulture.

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INVESTIGATIONS INTO THE RESISTANCE MECHANISMS OF THE GENUS RIBES AGAINST THE GALL MITE CECIDOPHYOPSIS RIBIS

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1. Introduction

The gall mite **Cecidophyopsis ribis** is the most serious pest in the black currant (**Ribes nigrum**), both directly by inducing a gall ("big bud") and indirectly by transmitting the disease of reversion. West-European black currant cultivars show a more or less high degree of susceptibility. The red currant (**Ribes rubrum**) and the gooseberry (**Ribes uva-crispa**) are resistant (Knight et al., 1974). Sometimes, red currant buds are infested by a specialized race of mites (Proeseler, 1973), but typical big buds are not formed. In breeding for gall mite resistance in the black currant, the gooseberry was chosen as the main donor (Knight et al., 1974), but the nature of this resistance is not clear (Anderson, 1971).

In the present paper the first results of the investigations into the resistance mechanisms are represented. Information was obtained

1) by studying the behaviour of the gall mite in laboratory tests during the free living phase of its life cycle in spring;
2) by studying the behaviour of the mites on hosts with different degrees of resistance
3) by analyzing the phenolic contents of the **Ribes** buds in order to find some correlation between the phenolics and the resistance or the susceptibility.

2. Experimental

2.1. The laboratory tests

The reaction of the migrating mites ot physical stimulants was tested in Petri dishes and on glass plates. All experiments were carried out in a room with constant climate (15°C, 14 h light, 10 h darkness).

For phototaxis experiments Petri dishes (9 cm in diameter) contained a little dish (3,5 cm in diameter) with a saturated salt solution (K_2SO_4 maintained a relative humidity of 99% in the atmosphere of the Petri dish). Three big buds were fixed by a thread at the wall of the dish (Fig. 2). The Petri dish was wrapped up entirely in paper leaving a light gap (1 x 1,5 cm) opposite the big bud.

The reaction to gravity was tested in a Petri dish standing vertically in a dark box. Three big buds were fixed in the middle of the dish. At a distance of 2 cm there was a stripe of glue to trap mites migrating to the top, to the bottom or to the sides of the dish (Fig. 4).

The hydrotaxis experiments. Two glass plates (7,5 x 2,5 cm and 6 x 2,5 cm) were stuck together with silicone rubber so that two channels were

formed between them. The upper ends of the channels were filled with cotton wool. The cotton in one channel was moistened with water, the cotton in the control channel remained dry. Near the lower ends of the channels two or three big buds were fixed (Fig. 5). The glass plates were put in a Petri dish standing upright.

2.2. Infestation experiments

In may five big buds form black currant cv. Rosenthals Schwarze Langtraubige were fixed by a thread on the new growth of several **Ribes** bushes which were planted in the garden of the institute in November 1984. The following cultivarş were used: red currants (Jonkheer van Tets, Rondom, Rote Holländische), black currants (Rosenthals Schwarze Langtraubige, Seabrook's Black), gooseberry (Weibe Triumph) and Josta (which is a double hybrid of black currants and gooseberries; Bauer, 1978). After one, two and three weeks two shoots respectively were examined of each cultivar under a stereomicroscope noting the occurrence of mites. For control non-infested shoots of a neighbour plant were examined in the same manner.

2.3. Analysis of the phenolic contents

The phenolic components of **Ribes** buds were extracted with methanol-acetone. The separation and analysis was carried out by use of column chromatography on polyamide, by thin-layer chromatography using cellulose as sorbent and by reversed-phase high performance liquid chromatography.

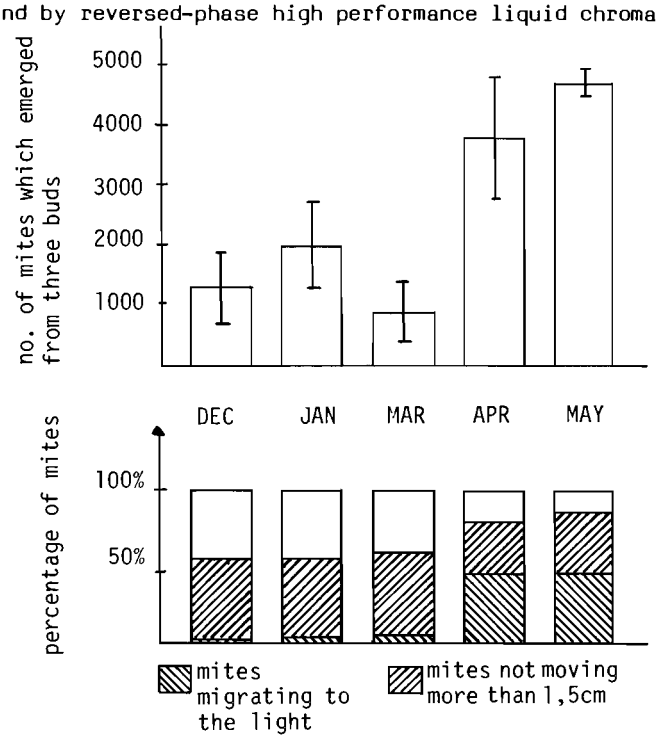


Figure 1. Results of the phototaxis experiment.

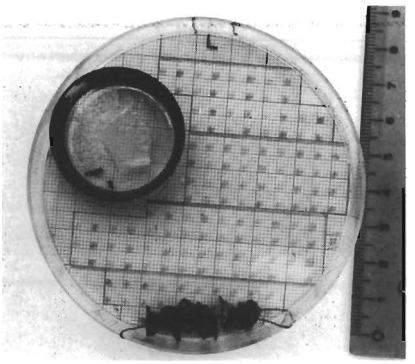


Figure 2. The phototaxis experiment

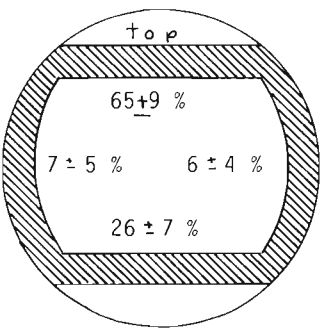


Figure 3. Reaction to gravity; percentage of mites migrating to the top, to the bottom or to the sides of the Petri dish

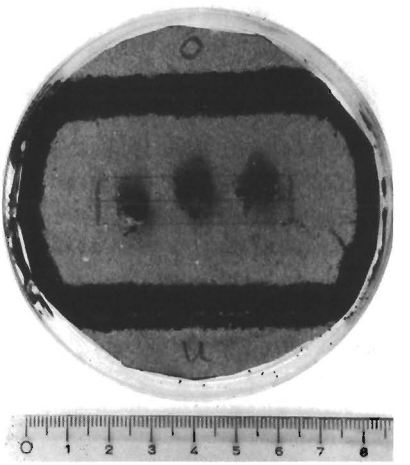
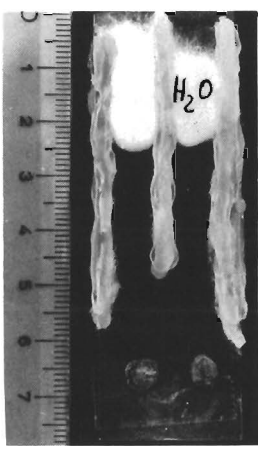


Figure 4. The geotaxis experiment



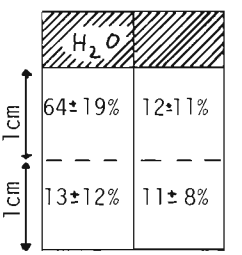


Figure 6. Reaction to humidity; percentage of mites, migrating to the moist cotton or to the dry cotton

Figure 5. The hydrotaxis experiment

3. Results and discussion

3.1. The laboratory tests

The results of the phototaxis experiment are shown in Fig. 1. From buds gathered in December, January or March only 1 - 6% of the emerged mites migrated to the light. From buds gathered in the middle of April or in May 50% of emerged mites migrated to the light. Taking into consideration that nearly 40% of emigrants did not move more than 1,5 cm from the bud, 80% of migrants (= mites moving more than 1,5 cm) crawled to the light. Thus the phototaxis experiment leads to two results. The first is the positive phototactic reaction of 80% migrating mites in May. The second is the different quantity and quality of mite population in different months. Quantitative population studies made by Smith (1961) and Collingwood and Brock (1959) had results with similar fluctuations in mite population as shown in Fig. 1. Beside this quantitative cycle qualitative differences exist: the mites leaving the buds before April have other qualities (for instance no positive phototactic reaction) than the mites leaving the buds during migration period in spring. For this reason the following tests for hydrotaxis and geotaxis were not made before May.

The results of the geotaxis experiment are shown in Fig. 3. Nearly 70% of migrating mites (emigrants that remained at the bud were not counted in this test) crawled to the top, about 25% of migrants crawled to the bottom of the vertically standing Petri dish.

The mites' reaction to humidity is shown in Fig. 6. The mites in the two channels were divided into two groups: those which crawled 3 - 4 cm up to the moist or to the dry cotton and those which crawled only 2 - 3 cm. About 65% of the migrants were found within 1 cm distance from the moist cotton (i.e. 3 - 4 cm above the big buds).

Gall mites emerge from big buds in early spring in order to penetrate a young bud in new growth (Smith, 1961). During this free living phase the positive phototactic and the negative geotactic reaction are useful in leading the mites to the new shoots. Their preference of humidity is a possible explanation why gall mites appear between the young leaves near the point of growth and at the base of the petioles near the young buds.

3.2. Infestation experiments

On the shoots of the susceptible black currant cultivar Rosenthals Schwarze Langtraubige several mites were found at the base of the petioles, on the surface of the young buds, under the outer bud scales and between the terminal leaflets. All mites were alive.

On the shoots of the cultivar Seabrook's Black, which shows some degree of resistance (Knight et al., 1974), living mites were only found between the terminal leaves, but no mites in the vicinity of the young buds.

Josta which is resistance to gall mite (Bauer, 1978) was different from the susceptible black currant. Living gall mites were found on the surface of new buds and between the terminal leaves, but not under the bud scales. The number of mites occurring on new growth was smaller in the case of Josta and Seabrook's Black than in the case of Rosenthals Schwarze Langtraubige.

On the shoots of black currants and Josta no remarkable amounts of dead mites were discovered. At the shoots of the three red currant cultivars and the gooseberry this situation changed. During one week examination living gall mites were found under outer bud scales and between young leaves. Two or three weeks after infestation a great many dead mites were lying under outer bud scales and between the terminal leaves. The youngest leaves as well as the outer bud scales were brown.

In the case of the gooseberry the situation was similar to that of the red currant except the number of mites: only a few mites were discovered on the new growth of gooseberry.

The gall mites infesting the red currants were not able to colonize the young shoots because they died after some days. According to the terminology of resistance by Painter (1968) the resistance mechanism of the red currants could be an antibiosis. The currant Seabrook's Black or the hybrid Josta with a more or less high degree of resistance were distinguished from the red currants and the susceptible black currant by the small number of mites discovered on the new growth. Thus the mechanism of resistance could be an antixenosis. Finally, on the infested shoots of the gooseberry only a few mites were discovered and some of them lying under bud scales were dead. A combination of antixenosis and antibiosis could thus determine the resistance of the gooseberry.

3.3 Analysis of phenolic compounds

In the phenolic contents of the susceptible black currant the flavonol-3-glykosides predominate. Within the flavonols, the IAA-oxidase-inhibitor quercetin outweighs the IAA-oxidase-activator kämferol tenfold. This fact could be important for the induction of gall-tissue by the mites. The phenolic contents of the resistant red currants and gooseberry is lower than that of black currants. Blue and blue-green fluorescent substances predominate and not the flavonol-3-glykosides. Quercetin outweighs kämpferol only two-fold.

The phenolic compounds may have a direct effect on the mite for instance by killing them under outer bud scales. However, they may have an indirect effect for example by preventing the formation of a gall. According to the infestation experiments in the genus **Ribes**, probably two or more mechanisms of resistance occur. The results of the biological observations as well as the possible physiological functions of the phenolics have to be taken into consideration when interpreting data of chemical analysis.

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RESISTANCE MECHANISMS IN RICE TO THE BROWN PLANTHOPPER, NILAPARVATA LUGENS (STAL)

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1. Introduction

The brown planthopper, **Nilaparvata lugens** (Stal) continues to be a serious pest of rice in South-East Asia. The initially successful use of resistant varieties as a control measure is now tempered by the ability of the insect to develop populations that can survive on previously resistant varieties (Claridge & Den Hollander, 1980) within a few years of their release (Pathak & Heinrichs, 1982).

A collaborative project between TDRI and the International Rice Research Institute (IRRI) in the Philippines is concerned with attaining durable resistance to brown planthopper through an investigation of resistance mechanisms.

In the initial stages of this project, the feeding behaviour of brown planthopper on susceptible (IR22), moderately resistant (IR46) and highly resistant (IR62) rice varieties was studied in detail. High-resolution video techniques were used to monitor behaviour over a four-hour period (Cook et al., 1986). Significantly more frequent, shorter probes an less honeydew were produced on IR62 and IR46 then on the susceptible variety, IR22, during the observation period. Additionally, there was no evidence from histological examination of stylet sheaths of a reduced ability to locate the phloem in the resistant varieties, suggesting that feeding was less on IR62 and IR46 largely as a result of one or more factors associated with the phloem, which may or may not be the same in both varieties. In order to provide further support for the phloem as the location of the major resistance mechanism(s), the work has been extended to record electrical activities of stylet penetration concurrently with video monitoring of behaviour of brown planthopper on these three varieties. The results are presented here.

2. Materials and methods

Insects. late instar nymphs of **N. lugens** were collected from susceptible rice variety TN1, grown at IRRI August-November 1985. The nymphs were transferred to potted TN1 plants and newly moulted adults were removed daily. For all observations, 4-30 hour-old, macropterous females were used.
Plants. Seedlings of IR22, IR46 and IR62 were transplanted into a field plot: 28-40-day-old tillers were used in experiments.
Electronic and video monitoring. The DC system used in this investigation was developed to monitor stylet activities of aphids (Tjallingii, 1978). An adjustable DC voltage source supplied an electrical potential (+/- 600 mV)

to the soil surrounding the roots of individual tillers via a copper electrode. For all observations a positive potential was applied.

One end of a piece of gold wire (Length, 5 cm; diameter, 25um) was attached to the insect's scutellum by conductive silver paint and the other was connected to a DC amplifier (input resistance 10^8 ohms). When planthopper stylets prenetrated the plant, electrical signals or electrical penetration graphs (EPGs) were monitored on an oscilloscope and recorded on an FM tapoe recorder and paper chart recorder (bandwidth 75 Hz).

Honeydew production was observed at the same time using an adjustable TV camera (Cook et al., 1986). Bromocresol-green indicator paper (Blackman, 1974) or plain filter paper was placed around the base of the tiller so that honeydew drops could be collected. Insects were monitored on the three varieties over a four hour period.

3. Results

More probes were made by insects on plants of IR46 (moderately resistant) than by insects on susceptible IR22, although there was no significant difference between the number of probes made into IR22 and highly resistant IR62 (Table 1). Fewer droplets of honeydew were recorded from insects on IR46 and IR62 than on IR22 (Table 1).

In EPGs of **N. lugens**, at least six patterns were of regular occurrence and these have been identified by their apppearance and frequency characteristics (unpublished results). Two of these patterns were associated with honeydew production; a 3-7 Hz pattern (Figure 1) and a low frequency pattern of 0.3-1 Hz (Figure 2).

The 3-7 Hz pattern, together with honeydew production, was observed from insects on all varieties and the mean total duration of this pattern was similar, approximately 40 minutes in the 4 h observation period (Table 1). This type of honeydew gave a light blue colour on bromocresol-green indicator paper.

Table 1. Feeding behaviour of **N. lugens** on three varieties of rice during a 4 h observation period (+/- S.D.).

	IR22	IR46	IR62
Mean number of probes. (EPG)	11.6(7.6)a	21.6(12.1)b	17.5(9.2)ab
Mean number of honeydew drops (observed)	5.3(5.9)a	0.8(1.3)b	1.7(2.4)b
Mean duration of 3-7 Hz pattern. (min)	40.0(39.0)a	36.7(24.4)a	42.2(30.0)a
Mean duration of 0.3-1 Hz pattern. (min)	81.4(67.3)a	26.2(36.1)b	15.8(20.4)b

Values within a row followed by different letters are significantly different $p<0.05$. IR22, n=20; IR46, n=19; IR62, n=20

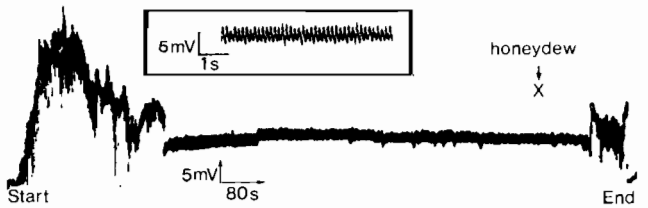

Figure 1. Electrical penetration graph (EPG) from **N. lugens** on rice variety IR46 showing 3-7 Hz pattern. Insect shows detail of pattern. 6 Hz.

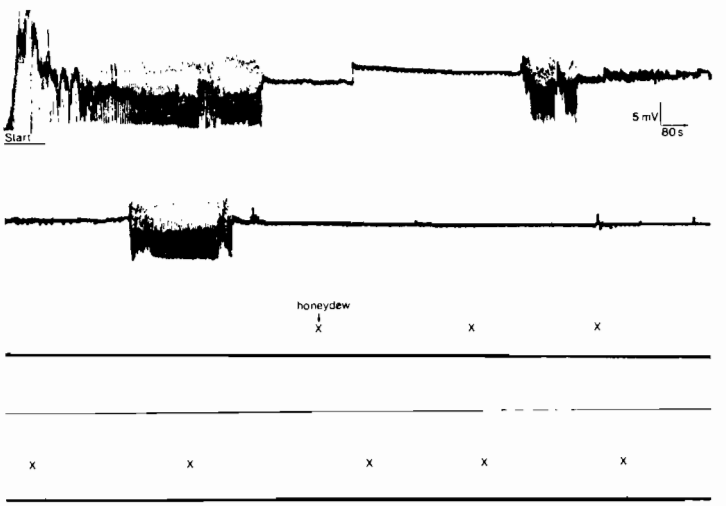

Figure 2. EPG from **N. lugens** on rice variety IR22 showing 0.3-1 Hz pattern.

The 0.3-1 Hz pattern was also recorded from insects on all varieties, but the mean total duration of this pattern was significantly lower from insects on IR46 and IR62 than from those on IR22 (Table 1). Also honeydew was only produced from insects on IR22 during this pattern in the 4 h observation period. This honeydew left a dark-blue spot on bromocresol-green paper.

4. Discussion

The results concerning the two patterns associated with honeydew production are summarised in Table 2 and it is suggested that they relate to feeding on different tissues in the rice plant.

The reaction of the honeydew with bromocresol-green paper indicates that the honeydew from the two patterns have different pHs. It has been shown in recent work that the honeydew associated with the 0.3-1 Hz pattern has a higher concentration of amino acids than honeydew excreted during the 3-7 Hz pattern (D.E. Padgham, unpublished results). Sogawa (1973) reported that **N. lugens** feeds primarily from the phloem of rice plants. Phloem sap has a relatively high concentration of amino acids and a higher pH compared to the sap of surrounding tissue (Moorby, 1981). Therefore the honeydew produced during the 0.3-1 Hz pattern may originate from phloem ingestion. This pattern occurred in the EPG's of insects on all varieties but the total duration was significantly lower on resistant varieties than on the susceptible variety and honeydew was never produced during the 4 h observation period. It is possible therefore that one or more factors in the phloem of the moderately resistant and the resistant varieties deter sustained ingestion.

The honeydew associated with the 3-7 Hz pattern may originate from non-phloem sources. Other homopterans such as the rice green leafhopper, **Nephotettix cincticeps**, ingest from the xylem tissues (kawabe, 1985). The xylem conducts water and ions through the plant and lies close to the phloem cells (Moorby, 1981). This tissue may be the source of the honeydew produced by **N. lugens** during th 3-7 Hz pattern.

To substantiate these proposals the observations from the monitoring system will be combined with the histological examination of saliva sheaths to provide information on stylet positions during the two patterns. It is also necessary to show that the patterns are associated with actual sap uptake, at least up to the gustatory receptors in the pharyngeal cavity (Foster et al, 1983). This could be shown by combining the monitoring system with stylet amputation (Kimmins & Tjallingii, 1985).

It is important to stress that the monitoring technique requires the use of tethered insects and the paint and wire may severely affect the insect's behaviour (Tjallingii, 1986). Tethered insects on IR22 made more probes and produced less honeydew than untethered insects on the same cultivar in previous experiments (Cook et al., 1986). The effect of tethering on behaviour will be assessed from simultaneous observations of tethered and untethered insects.

From the EPG data recorded here it is clear that both xylem and phloem tissues are located by **N. lugens** on all three varieties of rice. The differences lies in the amount of time spent in the 0.3-1 Hz (phloem)

pattern. This supports the proposal that resistance mechanisms in IR46 and IR62 are related to the phloem. Some ingestion probably also occurs from the xylem but this is apparently unrelated to resistance in these cultivars.

Table 2. Two patterns associated with honeydew production (4 hr observation period).

	1) 3-7Hz pattern	2) 0.3-Hz pattern
a	Pattern produced by N.lugens on all varieties.	Pattern produced by N.lugens on all varieties.
b	Duration of pattern was similar on all varieties.	Duration of pattern was significantly greater on susceptible variety, IR22 than on moderately resistant and resistant varieties.
c	Honeydew was produced from all varieties.	Honeydew was produced only from susceptible variety IR22.
d	1st drop on bromocresol green paper: pale blue	1st drop on bromocresol green paper: dark blue.
	Honeydew from xylem?	Honeydew from phloem?

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OVIPOSITIONAL RESPONSES OF HELIOTHIS SPP TO HOST PLANT VARIATION IN COTTON (GOSSYPIUM HIRSUTUM)

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1. Introduction

Two species of noctuid moths, **Heliothis armigera** (Hubner) and **H. punctigera** Wallengren, are the major pests of cotton in Australia. Both are highly polyphagous, having been recorded from a total of 161 plant species in 49 families, including most cultivated crops. Although much is known of their seasonal patterns of host use (Wardhaugh et al., 1977) little is known of the behavioural mechanisms of host selection by **Heliothis** spp.. Many factors such as surface texture (Cullen, 1969), the presence of flowers and nectar (Adjei-Maafo et al., 1983), plant volatiles (Hedin, 1976) and surface chemicals (Jackson et al., 1984) all appear to influence the selection and acceptance of oviposition sites, but their broad host range suggests that females may not be highly discriminating.

In Australia attempts to enhance the resitance of cotton to **Heliothis** have concentrated on four morphological traits; glabrousness, nectarileŝs. frego bract and okra leaf (Thomson & Lee, 1980) which variously influence its attractiveness to pests. Here I examine the responses of **Heliothis** females to two of these traits; glabrousness and okra leaf. Normal cultivars of **Gossypium hirsutum** are covered with trichomes over the surface of leaves, on stems, petioles and bracts. In glabrous genotypes trichome numbers are greatly reduced. Here the comparison was between glabrous ultrasmooth types and the delta smooth types characteristic of the current commercial cultivars. These have trichomes on the principal leaf veins, leaf margins and stems and are visibly more hairy than glabrous types. Okra leaf phenotypes have a deeply lobed leaf blade which results in a 35% reduction in leaf area a relative to normal broad-leaved cultivars, thus producing a more open canopy.

In addition I present some preliminary results from experiments which examine the discriminating ability of females in response to other sources of plant variation which may directly influence larval survival; the numbers of fruit per plant and the presence of feeding larvae on plants.

2. Materials and methods

2.1. Field experiments

Eight near-isogenic lines comprising all combinations of the glabrous, frego bract and okra leaf characters were developed in order to examine their impact on agronomic properties and insect pests (Fitt et al., in prep). Small plot experiments were conducted over three seasons (1983/84, 84/85, 85/86). Isolines were sown in a randomised block design with 4

replications. Each plot measured 20 rows x 20 m.

The number of eggs/plant and their distribution among structures was recorded twice weekly on 1 metre of row per plot (i.e. 8 m/genotype/week) throughout the growing season. Seven categories of plant structure were recognised as oviposition sites: upper and lower surfaces of young leaves (UYL, LYL), upper and lower surfaces of old leaves (UOL, LOL), squares (SQ), growing tip (TIP), and stem (STEM). Old and young leaves were distinguished by colour, surface texture and the degree of blade expansion. The category SQUARE includes other fruiting structures such a as open flowers and small green bolls, while TIP refers to unexpanded leaves surrounding the growing terminal.

Measurements of egg density and within-plant distribution were also made on two cultivars arising from the breeding program; Sicot 3 (glabrous) and Siokra (okra leaves), which were grown in commercial blocks (50-100 ha) adjacent to similar sized blocks of the normal leaf cultivars used commercially; Deltapine 61 and Deltapine 90.

2.2. Field Cage Experiments

Oviposition behaviour was assayed in large outdoor screen cages (5 m x 3 m x 3 m) using potted plants (2 plants per pot). Plants were used for bioassays at about the time of first flower when they were approximately 60 cm high. Eight groups of plants, each consisting of three pots (i.e. 6 plants/group), were arranged in a 4 x 2 grid with 1.5 metres between adjacent groups. Each experiment involved a choice between two alternatives which were arranged at alternate grid positions. The following choices were tested: ultrasmooth vs delta smooth, okra leaf vs normal leaf, clean plants vs plants with feeding larvae, plants with intact squares vs plants with squares removed.

Moths for behavioural assays were either collected as larvae from the field and reared to pupation on an artificial diet at 25°, or else were the F1 laboratory progeny obtained from such moths. For each experiment 10-20 pairs of sexually mature moths were released into the cage late in the afternoon. No adult food was provided. Other than that available at the nectaries of the cotton plants. Each experiment ran for two nights, with the treatments rearranged among grid positions each day and each experiment was repeated from 2-4 times using new batches of moths and plants. The number of eggs laid on each plant and their distribution among plant structures was recorded daily. All eggs were removed during counting. Data on the numbers of eggs/plant was analysed using two or three factors ANOVAS after x+1 transformation to normalise the variances. Deterrence indices based on the numbers of eggs on control (B) and test (A) plants were calculated as : DI = (B-A)*100/(A+B) (Renwick & Radke, 1985).

3. Results

3.1. Responses to Glabrous and Okra-Leaf Characters

3.1.1. Egg densities. In field cage experiments, **H. punctigera** consistently preferred the deltasmooth isoline to the ultrasmooth glabrous isoline (Table 1). Observations of 12 females over two nights showed that while the toatl number of approaches to the two isolines were similar,

females remained on glabrous plants for shorter periods and deposited fewer eggs/visit than on the hairier genotype (Table 2). By contrast neither species showed a significant response to okra leaf relative to the normal leaf shape (Table 1).

In the small plot field experiments, which relied solely on oviposition by the natural **Heliothis** population, the influence of the two leaf characters differed between seasons (Table 3). In the 1985/86 season, when oviposition was greater overall than in other seasons, the glabrous character led to a significant reduction in egg numbers of about 36% (Table 3). In the 1983/84 season the glabrous character also had a significant effect, due primarily to a significant interaction with okra leaf. Apart from this interaction the okra leaf character had little effect on egg densities.

Despite the variable results in small plots, cultivars incorporating the two leaf characters (Sicot 3 - glabrous, Siokra - okra leaf) grown in commercial blocks have shown significant effects on **Heliothis** oviposition. At one site, Sicot 3 averaged 40% fewer eggs than DP61 ($P<0.05$) over a season (mean eggs/check: Sicot 3 - 2.99 eggs/m, DP61 - 4.95 eggs/m). Similarly at 3 sites in 1985/86, Siokra showed a significant reduction ($P<0.05$) in egg numbers relative to the normal leaf DP90 of about 25% (mean eggs/check: Siokra - 5.03 eggs/m, DP90 - 6.80 eggs/m).

Table 1. Discrimination by female **Heliothis** between Glabrous and Deltasmooth or Okra leaf and Normal leaf Isolines in field cages.

Species of *Heliothis*	Plant Genotype	Mean Squares/ plant	Mean Eggs/ plant	% of eggs	Deterrence Index
H. punctigera	NORMAL	4.28 a	85.71 a	66.10	
	GLABROUS	4.50 a	43.95 b	33.90	32.2 ***
H. armigera	NORMAL	5.11 a	19.95 a	52.33	4.7 ns
	OKRA	6.67 b	18.17 a	47.67	
H. punctigera	NORMAL	5.28 a	11.58 b	47.66	4.7 ns
	OKRA	6.89 b	12.72 b	52.34	

Means in each column followed by same letter are not significantly different at P=0.05. *** P<0.001

Table 2. Behaviour of female **H. punctigera** in response to Glabrous and Deltasmooth isolines in field cages.

Genotype	Mean no. approaches	Mean time / bout (secs) Oviposition	Feeding	Mean eggs /group
NORMAL	43.2 a	85.0 a (15)*	113.5 a (14)	183.3 a
GLABROUS	38.7 a	26.0 b (12)	73.4 b (14)	81.8 b

* Number timed bouts. Letters indicate significant differences at P=0.05.

3.1.2. Within-plant egg distribution. The two morphological characters differed in their influence on the wihtin-plant distribution of eggs. Glabrousness had no significant influence, either in field cages or in the field. The majority of eggs (n = 1769) were laid on the upper surfaces of

Table 3. Cumulative densities of **Heliothis** eggs on small plots of 4 cotton isolines with differing leaf shape and hairiness over 3 seasons.

Season	Mean eggs/m (maximum)	Cumulative egg density GENOTYPE NN	NO	GN	GO	Sign. Main effects	Sign. interactions
83/84	0.55 (2.40)	7.78 a	12.58 b	7.53 a	5.63 c	Hair ***	Hair x leaf *
84/85	0.71 (4.15)	16.89 a	16.81 a	17.89 a	13.99 a	-	-
85/86	2.22 (10.30)	77.19 a	74.31 a	43.46 b	53.33 b	Hair ***	-

N=normal leaf or hair, O=okra leaf, G=glabrous. * P<0.05, *** P<0.001

young (63.5%) and old (38.0%) leaves. By contrast the okra genotypes received fewer eggs on young leaves and showed an increase in the proportion of eggs laid in fruiting structures (Table 4).

Table 4. The distribution of eggs by **Heliothis** spp. among plant structures of Siokra (Okra leaf) and DP90 (Normal leaf) in the field.

Variety	Species of *Heliothis*	Plant Structure UYL	LYL	UOL	LOL	SQ	TIP	STEM	Total eggs
DP 90	*H. a* %	31.1	3.1	40.6	3.1	16.7	2.7	2.7	711
	H. p %	32.7	10.5	20.5	4.2	14.5	9.6	8.0	801
SIOKRA	*H. a* %	24.5	2.8	38.0	3.5	25.7	2.4	3.0	460
	H. p %	25.6	8.5	18.7	2.3	24.9	12.4	7.6	727

H. a - *H. armigera*, *H. p* - *H. punctigera* Total eggs 2699

3.2. Responses to the numbers of fruit and presence of feeding larvae

When offered a choice between groups of plants bearing a mean of 29.8 squares or similar plants from which all squares had been removed, both **Heliothis** species showed a significant preference for plants with intact squares (**H. armigera:** 34 fewer eggs on desquared plants, P = 0.042; **H. punctigera:** 60% fewer eggs, P = 0.011).

When offered a choice between undamaged plants and plants on which larvae had fed for 24 hours and were still present (10 3rd instars/6 plants), female **H. punctigera** showed a significant preference for the undamaged plants and laid 66% more eggs on these than on the infested plants (P<0.001). This response was independent of plant genotype.

4. Discussion

The generalist host acceptance behaviour of **Heliothis** spp. has hampered the development of commercially useful insect-resistant cotton cultivars. While in small plot or field cage choice experiments females may discriminate against particular cultivars, this advantage often disappears when the same cultivars are grown extensively. Without the opportunity to discriminate females may readily accept less preferred cultivars.

Several studies have shown reduced **Heliothis** egg laying on glabrous cottons (e.g. Likefahr et al., 1971; Davis et al., 1973). However, there has been no attempt to identify the mechanism of discrimination involved. Cullen (1969) found that surface texture (hairiness) was the major post-

alighting cue involved in stimulating oviposition of **H. punctigera** on lucerne. The responses of females to glabrous cotton reported here strongly suggest a post-alighting discrimination to tactile cues from the plant surface. Whether the glabrous character also influences the distance moved between plant visits or the tendency of females to leave a patch is not known.

While the okra leaf character confers resistance against some pests (e.g. white fly and mites, Brettel, 1980), it has not ben seen as a direct mechanism of resistance against **Heliothis**. However the more open canopy allows better penetration of insecticides and this is thought to confer better control of larvae. In this study the reduced oviposition on okra-leaf cultivars in the field was due mainly to **H. armigera** (Fitt, unpub.). This discrimination, though not evident in field cage trials, may result from the reduction in leaf surface area of okra plants coupled with a greater tendency of **H. armigera** females to lay on the upper surfaces of older leaves (Table 4). On the other hand the increased proportion of eggs laid directly on squares of this genotype, perhaps due to fruiting structures being more exposed, could result in an increase in early larval survival.

The experiments also showed that female **Heliothis** are capable of discriminating between plants in response to the presence of fruiting structures of feeding larvae. In the field females show a preference for the phenological stages of maximum square production (Adjei-Maafo & Wilson, 1983) probably in response to changes in the production and composition of volatiles released above the crop (Hedin, 1976). However, in the experiment described here the plants were identical except that half had all squares removed prior to testing. The reduction in eggs laid on these plants may represent a response to the physical absence of fruit, even though these are not the most preferred oviposition sites. Alternatively it may result from the absence of specific volatiles produced only by the squares (Hedin,. 1976) or to a rapid change in volatile production by the damaged plants. The avoidance of plants with feeding larvae is common to many Lepidoptera and other insects. However, there is no evidence for the use of oviposition-deterring pheromones (Prokopy, 1981) by **Heliothis** nor do females avoid plants bearing only unhatched eggs (pers. obs.). While in other Lepidoptera the avoidance of infested plants is often due to endogenous volatiles released form larval frass (e.g. Renwick & Radke, 1980), preliminary experiments (Fitt, unpub.) suggest that in **Heliothis**, frass alone is not repellent.

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LOW MOLECULAR CARBOHYDRATES OF ZEA MAYS L. LEAVES AND THE EGG-LAYING OF OSTRINIA NUBILALIS Hbn. LEPID. PYRALIDAE

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1. Introduction

The European corn borer, **Ostrinia nubilalis** Hbn, lays its eggs on the under-side of leaves of a great number of plant species. Among cultivated plants, maize if often selected and within the species the insect is able to discriminate hybrids (Everly et al., 1979; Anglade et al., 1980). Different factors were assumed to be involved as genetic characters: pilosity and hybrid earliness (Andrews & Carlson, 1976), phenological stages (Everly, 1979), plant height (Burgstaller, 1974) and other undetermined characters which seem to be transmitted by inbred lines.

In fact a lot of stimuli may be simultaneously present in the environment of the insect. To this date, no biochemical explanation of the oviposition site choice of **O. nubilalis** have been given, whether on corn or on other plant species. Our studies were especially devoted to contact substances of corn leaves which could be detected by the European corn borer females and thus determine its choice. The insect is able to distinguish several corn hybrids which could be classified in three large groups according to the number of egg-masses deposited: suitable, unsuitable and intermediate for oviposition.

First results obtained from observations of suitable and intermediate whole maize hybrid plants led us to propose some biochemical interpretations. By comparing a suitable hybrid (AxF) with an intermediate one (LG11) it was suggested that a positive correlation exists between carbohydrate content of maize learves ans number of egg-masses laid on the plant (Derridj & Fiala, 1983); the choice is rather accurate and remains when the two hybrids are cultivated side by side alternately. In order to establish a better correlation between these substances and the oviposition, excluding genetic characters differentiating the hybrids, experiments were limited to the simple hybrid AxF. The purpose was to modify low molecular carbohydrate contents of parts of the plants and to give the insect a choice between these modified plants and the controls. We used two methods: reducing carbohydrate contents by a 24 h dark exposure of plants (Fiala et al., 1985), and enhancing these contents by treatment with maleic hydrazide, a systemic growth regulator (Derridj et al., 1986). In each case the insect chose the plant richest in sugars at twilight and the choice was more marked as both the number of sugars involved (glucose, fructose, sucrose) and their contents were greater.

These results obtained with a single hybrid corroborate our previous conclusions with two hybrids (Derridj & Fiala, 1983). Furthermore, when the

carbohydrate content was reduced by placing plants in the dark, the egg-mass distribution on stratum leaves was different from that observed on control plants.

The aim of this communication is to show that all these interpretations have been confirmed by new observations and by other types of experiments using artificial supports.

2. Studies with whole plants

Results of experiments with LG 11 and with a maize hybrid from the West Indies, showing a higher leaf carbohydrate content showed a marked correlation between low molecular carbohydrate leaf content and moth oviposition choice.

	Corn hybrid	8th leaf carbohydrate content (mg/g FW)			Egg-masses	
		glucose	fructose	sucrose	number for 100 plants	ratio
Field conditions	AxF	2.7	1.8	5.2	36.7	2.26
	LG 11	1.8	1.2	4.0	16.7	
Glasshouse conditions	West indian hybrid	4.8	1.7	11.5	633	6.30
	LG 11	2.7	1.5	7.9	100	

These recent results confirm the relationship which was previously established between low molecular carbohydrates of different maize hybrids and oviposition choice of the European corn borer (Derridj & Fiala, 1983; Fiala et al., 1985; Derridj et al., 1986).

Other experiments were conducted to study the variations of non structural leaf carbohydrate contents during egg-laying period including twilight and the beginning of dark period. Maize plants were cultivated in phytotronic conditions. Results concerning carbohydrate contents of the 7th leaf from the stem base at the 11-leaf phenological stage (in AxF) showed that the carbohydrate content remains rather stable during 3 hours of darkness. A lower carbohydrate level was observed after 6 hours of darkness.

Darkness (in hours) following 3 hours of twilight	Leaf carbohydrate contents (mg/g FW)		
	Glucose	Fructose	Sucrose
0	1.0	0.5	4.2
1.5	1.0	0.8	2.8
3	0.8	0.5	3.4
6	0.5	0.4	1.9

These results show that leaf carbohydrate contents at twilight are the same during the insect oviposition period. Moths lay their eggs when carbohydrate leaf contents are rather high. But another point should be clarified. Is there exudation of low molecular carbohydrates at the leaf blade surface?

3. Studies with leaf segments

In order to explain how the low molecular carbohydrates may act on the insect, an attempt was made to increase the content of these substances in maize leaves separated from the plant and to determine the relationship between carbohydrate content and oviposition. The two tips of portions of maize leaf blades 25 cm long were immersed into an aqueous 6% sucrose solution for different time periods; control leaf blades were immersed in distilled water. In each experiment two leaf stripes (control and 6% sucrose solution) were placed in small cages in a phytotronic chamber and the number of egg-masses was noted.

Type of choice	Carbohydrates contents (mg/g FW)			Eggs masses	Eggs
	Glucose	Fructose	Sucrose		
Control	0.3	0.3	2.8	89	1758
11 h 6 % sucrose solution	1.0	1.0	13.1	109	2184
χ^2 significance level				n.s.	$p < 0.01$
Control	2.4	0.8	1.6	19	327
24 h 6 % sucrose solution	4.6	2.4	9.3	43	835
χ^2 significance level				$p < 0.05$	$p < 0.01$
Control	0.2	0.2	2.1	30	598
31 h 6 % sucrose solution	8.1	6.3	38.5	45	946
χ^2 significance level				n.s.	$p < 0.01$

These results show that a greater number of eggs were obtained on carbohydrate enhanced leaf stripes, while the number of egg-masses remained unchanged.

Moreover it was observed that addition for 31 h of sucrose $^{14}C(U)$ containing the same total radioactivity (880 KBq) into these two solutions results in appearance of radioactivity at the leaf surface. This

radioactivity was higher at the surface of leaves immersed into 6% sucrose solution (228 Bq) than at the surface of leaves immersed into water (7 Bq). This suggests a direct relationship between the low molecular carbohydrates inside the leaf tissue and the same carbohydrate on the leaf surface and might explain their influence on oviposition choice.

4. Studies with artificial support

The preliminary experiments reported hereafter show that three low molecular carbohydrates (glucose, fructose and sucrose) are present on maize leaf surfaces at contents ranging from 10^{-5} to 10^{-6} moles per m^2 leaf surface area. We observed a marked increase of carbohydrate contents on leaf surface after treatment with maleic hydrazide; the quantity of each carbohydrate was multiplied by 8,4 and 3 respectively, as compared to levels found in the untreated plants. When Whatman paper stripes were imbibed with these sugar concentrations and submitted to the insect's choice, results on the 3rd day of oviposition were: 15 egg-masses and 239 eggs on the poorer paper and 29 egg-masses and 503 eggs on the richer one.

Further preliminary results obtained showed the complex and apparently simultaneous action of these three sugars suggesting that relative quantities of these carbohydrates seem to determine the insect response.

5. Conclusions

The choice made by the insect depends on its sensorial and behavioural capacities to discriminate its environment. All the stimuli are associated. By the methods used here, we tried to integrate as many stimuli as possible and then to isolate them. The correlation between carbohydrates and oviposition of **O. nubilalis** is more obvious on the whole plant than on supports. Many factors present at twilight might be involved and better knowledge of the host plant itself may provide further explanations.

Nevertheless **O. nubilalis** is a scarce example in which oviposition behaviour would be regulated in part by chemical stimuli as nutrient which is also involved in larvae feeding (Robert, 1986). The high discrimination of the female to recognize by contact the support richest in carbohydrates, and the suitability of this choice for the progeny is an illustration of maintenance of an insect owing to its polyphagia based on essential nutrients. It is of great interest, after having shown this discrimination between favourable or intermediary corn hybrid for oviposition, to examine the incidence of carbohydrate content of unfavourable hybrids and finallly to extend the research to the choice of the insect between several species of wild and cultivated plants.

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STYLET PENETRATION ACTIVITIES BY APHIDS: NEW CORRELATIONS WITH ELECTRICAL PENETRATION GRAPHS

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1. Introduction

Light microscopic studies suggest that aphid stylets follow and intra- and/or intercellular pathway to the phloem (Pollard, 1973). Spiller et al. (1985), and others indicated that resolution of the electron microscope (EM) is needed for location of stylets in relation to cell structures. General occurrence of intercellular penetrations by hydrolysing the middle lamella, as assumed by McAllan & Adams (1961), is not suppported by this EM evidence. Neither it was by data from electrical penetration graphs (EPGs). From aphids which were supposed to penetrate intercellularly, according to the latter authors, no lower numbers of intracellular punctures were recorded than from other aphids (Tjallingii, 1985b). These stylet punctures are recorded as clear drops of the electrical potential (potential drop; pd) due to the membrane potential of plant cells. This knowledge and improvements of the EPG amplifier (Tjallingii, 1985a) allowed further details of wave-form patterns to be distinguished. Pattern E and F were initially described as 'additional to D'. On diet pattern D, including E and other D-associated patterns, was correlated with active ingestion (Tjallingii, 1978). Later, in pattern E on plants a distinction could be made between an extracellular E pattern (E(c)) and an intracellular E pattern (E(pd)). Stylet tips were located in a sieve element during sustained (E(pd)), which suggests relation to mainly passive ingestion (Kimmins & Tjallingii, 1985).

This paper concentrates on new correlations of pattern E(c), F, and other D-associated patterns with penetration activities.

2. Materials and methods

2.1. Aphids and plants used were **Brevicoryne brassicae** (L.) on Brussels sprouts, **Acyrthosiphon pisum** (Harris), **Aphis fabae** Scop., and **Megoura viciae** (Bukton) on **Vicia faba**, and **Myzus persicae** (Sulzer) on **Brassica napus**. For stock cultures aphids were reared on potted plants at 18 ± 3°C with 16 h light per day.

2.2. EPG recording includes attachment of a thin wire to the aphid dorsum and connection to an amplifier to which also the plant or diet is connected. The system (Tjallingii, 1985a) allows adjustment of the circuit potential; i.e. the plant- or diet voltage. Signals have two origins: (1) resistance fluctuations of the aphid which modulate the circuit potential and, (2) electromotive forces (emf) generated by the aphid or plant.

Resistance components depend on the magnitude and sign of the circuit potential, whereas an emf component is in principle independent of the circuit potential. Emf components of studied patterns were recorded by adjusting the circuit potential to zero Volts. Positive and negative adjustments caused recording of both components, resistance and emf superimposed; amplitudes either reinforced or counteracted each other. The amplifier had an input resistor of 100 Mohm but also a new type was used with a 1 Gohm input resistor, which provides a better emf responsiveness (Tjallingii, in press). EPGs were put on tape (FM recorder) and on a chart recorder (0-75 Hz).

2.3. Stylet location in the plant was determined during F. **M. viciae** was allowed to penetrate on sprout leaves (non-host) until F appeared. Then the stylets were rapidly cut and the piece of leaf containing the stylet stump was processed for EM (Kimmins & Tjallingii, 1985).

2.4. Fluid uptake during F was studied by measuring radioactivity of whole aphids (Tjallingii, 1978) after EPG recording on a 4.5 cm stem piece of **V. faba** loaded previously with 0.5 mCi ^{32}P (Amersham, PBS 13) in 0.4 ml 1.25 mM EDTA. Contaminated legs, antennae, and labium with stylets were removed. Honeydew was not produced. Uptake could only be expressed in terms of counts per minute (CPM), not in volume.

2.5. Fluid uptake during E and G, a newly distinguished pattern, was measured on diet by using a flow chamber. Disposable flow chambers were made out of soft polyethylene caps (o = 6.5 mm) by filling them with resin for the connection of two hypodermic needles; one for fluid supply the other as a drain. At the surface, between the two needle openings, a groove of cap materials was cut away to form the flow chamber when covered by a Parafilm membrane. The flow chamber was filled with 'cold' diet and mounted with the supply needle on a syringe with ^{32}P (5 mCi/ml) diet leaving an air bubble between the two fluids to prevent mixing. A wired aphid (o = .1 mm copper wire) was allowed to penetrate the chamber until E or G was showed. Then labeled diet was gently forced into the chamber until the air bubble appeared at the end of the drain. Radioactivity of aphids and pattern durations were measured.

3. Results

3.1. Pattern F was never observed on artificial diet. Starting after a bried period of A, B, and C (ABC), it could last from several minutes to hours, and it was either followed by pattern C or stylet withdrawal (Fig. 1). F did not occur in all penetrations and generally not in those that led to the phloem (sustained E(pd)). All aphid species studied showed F, on all plants tested. **M. viciae** showed more F on sprouts than on **V. faba**, as is found also for **A. pisum** (Tjallingii, 1986).

The electrical properties of patterns include frequency, shape, electrical origin, and the level of the signal potential: i.e. an intra- or an extracellular level. The frequency of F was rather constant within individuals and species. They ranged from 10 to 20 Hz; 10-13 Hz for **M.**

persicae and **B. brassicae** and 14-16 Hz for **A. pisum**. The shape of F is rather sinusoidal with sometimes flatened peaks (Fig. 2, inset). Its origin is due to resistance changes and to emf. The shape of both components is similar but the emf component often showed a periodical (1 per sec.) amplitude reduction (Fig. 2, trace 0). The potential level of F was always extracellular. Occasionally the level showed fluctuations caused by periods of increased resistance, not by emf from membrane potentials.

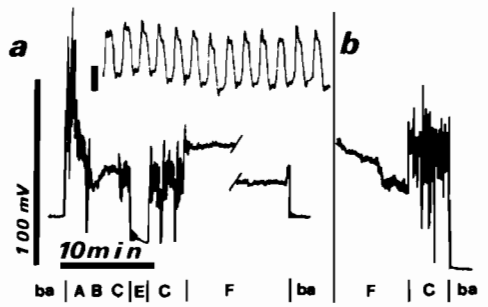

Figure 1. Two EPGs with F. Patterns indicated below traces: ba = base line; E = E(pd). a. With abrupt end, middle part left out; **B. brassicae** on host; inset 1 sec. of F; bar, 1 mV. b. Last part, with end after return to C; **A. pisum** on non-host.

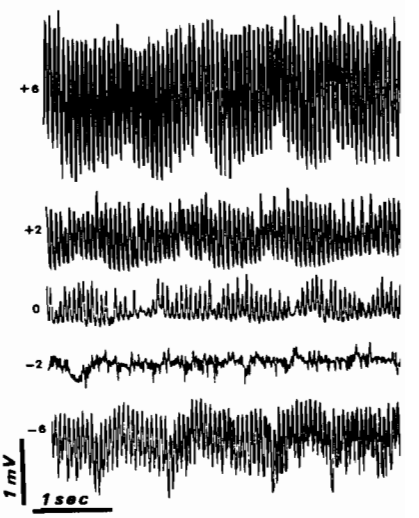

Figure 2. Pattern F from **A. pisum** on host at different plant voltage adjustments: 0, emf component; +6, maximal reinforcement of emf and resistance component; -2, maximal counteraction.

EM sections, after stylet cutting during F, showed the stylets in cell walls over long distances. Stylets were enclosed by a slender sheath and the separate tips were inserted differently deep (Fig. 3a). Most stylets were found in thick epidermal walls without a clear middle lamella. Thinner cell walls often showed traces of rupture and presumable repair. Mentik et al., (1985) found a number of stylet tips from **Nasonovia ribisnigri** on lettuce near the phloem (but not in sieve elements), which suggests that our found preference for epidermal tissue is due to the strange aphid-plant combination.

Radio-isotopes were ingested form ^{32}P loaded **V. faba** stem pieces by **M. viciae** during ABC and F. Aphids with ABC only showed a positive correlation between uptake and ABC duration (Spearman $R = 0.61$, $P<0.005$, $n = 21$). ABC uptake (CPM) was subtracted from the uptake of aphids with both ABC and F. The mean rate of uptake during F was about ten times higher than during ABC.

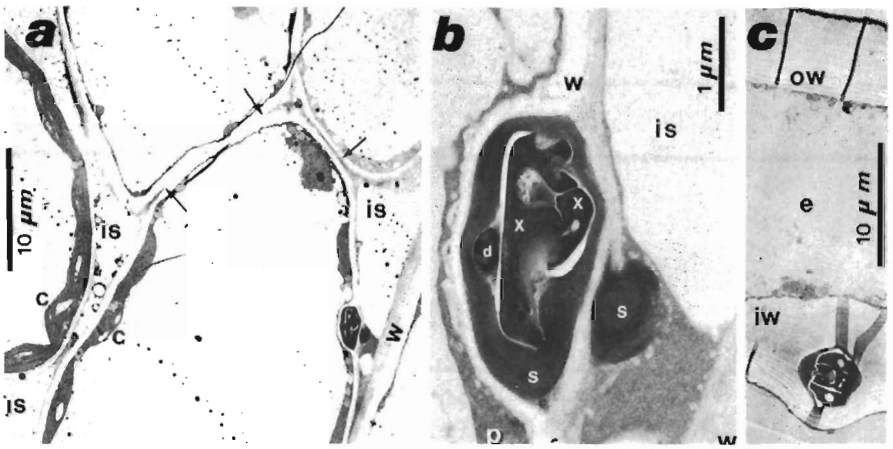

Figure 3. Stylets of **M. viciae** in cell walls of sprouts during F. a. Stylets in single wall of mesophyll cell. b. Detail of a, saliva entered through break into intercellular space, stylets inserted differently deep. c. Penetration of epidermal cell wall; dark cross bands are artefacts from section folds. Legend: ch, chloroplast; d, mandibula; e, epidermal cell; is, intercellular space; iw, inner wall; ow, outer wall; p, protoplast; s, saliva; w, cell wall; x, maxilla; arrows, middle lamella.

3.2. Two other D-associated patterns were studied in relation to ingestion; E and G. Patterns E has been described previously (Tjallingii, 1978a) as occurring superimposed on D (indicated as "D+E"; Kimmins & Tjallingii, 1985; Tjallingii, 1985a, b and 1986). Signal details showed, however, that D does not exist in itself but represents a number of patterns with a small amplitude and a certain DC-voltage. These patterns are E(c), E(pd), F, and G at the moment. E was studied on artificial diet; thus, a distinction between E(c) and E(pd) could not be made.

E showed, beside the earlier described 20 ms peaks at a rate of 0.5-3/s, some smaller positive pulses at a rate of 2-9/s (Fig. 4); 2-6/s in **M. persicae** and **B. brassicae** and 6-9/s in **A. pisum**. Plant or diet voltage adjustements showed that the small waves are of emf origin and the E-peaks have only a small negative emf component (Fig. 4, middle trace) but are mainly caused by resistance fluctuation; as is shown by their altering sign (other traces). On plants the small emf-waves were found during both E variants, E(c) and E(pd).

G is a new pattern, which is produced occasionally on diets and on plants, where it had only an extracellular potential level. It maximally lasted one hour. Its wave-form (Fig. 5) is sinusoid, often accompanied by sharp peaks when the plant or diet voltage is adjusted positively or negatively. The sinusoid frequency changes from 4 to 6 Hz, about the same as the sharp peaks which mostly occur at irregular intervals. The sinusoid has an almost exlusive emf origin but the sharp peaks have a major resistance component. G differs from E by (1) a more sinusoidal shape with (2) a much higher amplitude than the small waves, (3) a higher less regular

frequency of peaks if present, and (4) a smaller emf component of peaks.

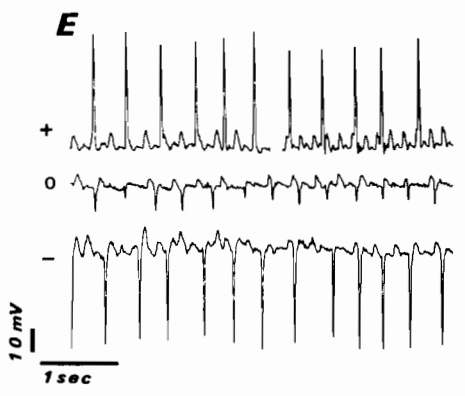

Figure 4. Pattern E at three adjustments. Two frequencies of small emf-waves shown with one of large peaks: + trace left and right. **B. brassicae** on host.

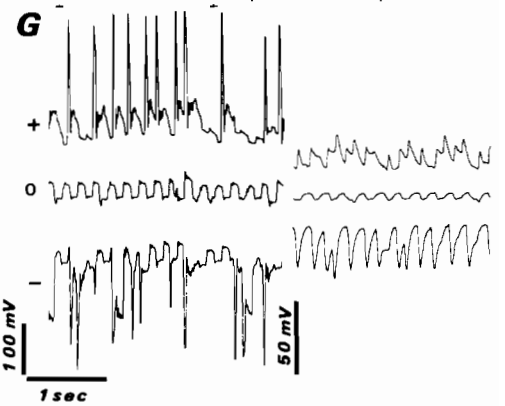

Figure 5. Pattern G at three adjustments. Large peaks in traces at the right are nearly absent. Left, **B. brassicae**; right, **A. fabae**, both on host.

If artificial diet in a flow chamber was replaced by an unpleasant fluid such as e.g. 0.1 N NaOH while **M. persicae** had been showing G, the aphid stopped G or withdraw its stylets. However, after replacement during E, the aphid continued with this pattern. This suggested that during E the replaced fluid did not reach the gustatory pharyngeal organ (Wensler & Filshie, 1969). In experiments with replacements by labeled diet, G showed the largest mean rate of fluid uptake (3.49 pl/s, n = 29) but ingestion was found also during E (1.01 pl/s, n = 25) and ABC (0.11 pl/s, n = 4). The rate of uptake during ABC found earlier (Tjallingii, 1978) was lower (0.2 pl/s, n = 20). Ingestion rates of E were positively correlated with the small emf-wave frequency (Spearman R = 0.56, $P<0.005$, n = 21) but no correlations were found with other wave-form parameters of E and G.

4. Conclusions and discussion

Active ingestion has been proved for three D-associated patterns: G, E and F in this sequence of importance. For F a 0.2-1 pl/s is obtained from the F/ABC ratio and an ABC rate of 0.02-0.1 pl/s. The significance of G has been recently elucidated by EM evidence for xylem position of the stylet tips, and increased occurrence of this pattern after more than 24 h starvation (Spiller & Tjallingii, in prep.). These data suggest that G is more related to active drinking than to feeding. Its general occurrence in all aphid species studies implies that G reflects a common penetration activity.

Active ingestion during E is limited but higher than was inferred from the deterrent fluid experiment. The small emf-waves, which were not yet discovered at the time of the experiment, need a further analysis in this respect as is shown by their correlation with the ingestion rate. Also, it remains unclear whether they are equally involved in E(c) and E(pd).

The stylet position as found during F seems an illustration of the 'classical' idea of an intercellular pathway. However, this pathway occurs only occasionally, does not lead to the phloem, and the middle lamella, and so pectinase, is hardly involved. Plants and in particular cell walls are a condition for the incidental occurrence of F. Its function remains unclear.

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THE EFFECTS OF INSECT HERBIVORES ON THE GROWTH AND REPRODUCTIVE PERFORMANCE OF ENGLISH OAK

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1. Introduction

The results presented here form part of a long term study into the direct and indirect effects of vertebrate and invertebrate herbivores on growth and recruitment of English oak, **Quercus robur**. The study consists of two parts: 1) a manipulative experiment in which all invertebrates are excluded by a programme of insecticide application; and 2) a larger, observational study aimed at discovering the effects of both vertebrate and invertebrate herbivores on plant performance. I shall restrict discussion in this paper to invertebrate herbivores, and to the results from the first 4 years of the observational study. Experimental results from the same period have been published elsewhere (Crawley, 1985).

At the beginning of the study, conventional wisdom suggested that the impact of insect herbivores on the growth of hardwood trees was typically rather low. Average levels of defoliation lay around 5 - 15% of leaf area per year, and these levels of defoliation were thought to have no detrimental effect on tree growth (largely because of plant compensation; see Crawley, 1983). Some dissented from this view. For example, Varley & Gradwell (1962) suggested that low density insect herbivores may be responsible for substantial losses of wood increment. This assertion, however, was based on a distinctly questionable linear extrapolation, and has not been verified in the experimental part of this study. It does appear, then, that low levels of defoliation have little impact on timber production. The tendency developed, therefore, to assume that since low density herbivores had no effect on tree growth, then they had no effect on the trees at all. This view has been shown to be false. Our experimental results demonstrate unequivocally that sprayed trees produce significantly more acorns, even at these low levels of defoliation (between 2 and 4.5 times the number produced by unsprayed trees). (A test of possible stimulatory effects of the insecticide on acorn production was afforded in 1985 when defoliation levels on the experimental trees were unusually low. In several cases the unsprayed trees produced more fruit than their sprayed counterparts; if the insecticide had been stimulatory, they would have produced less.)

The observational part of the study involves 36 tres ranging in girth from 0.38 to 7.48 m at breast height. Each year a random sample of 40 shoots is selected at two heights and two aspects and the following measurements taken for each individual shoot: primary shoot length; lammas shoot length; defoliation (see Crawley, 1985, for method of determination);

number of peduncles; and (per peduncle) the numbers of acorns, galls of **Andricus quercuscalicis**, small and aborted acorns. Early in the year, numbers of male and female flowers per shoot are estimated, along with the numbers of buds on primary and lammas shoots which remain unopened (so-called 'back-up' buds which may burst following heavy early defoliation).

2. Results and discussion

2.1. Acorns

Our pilot study suggested that some trees produced heavy acorn crops every year, while others produced consistently light crops. excluding trees that are too small to produce any female flowers (oak trees tend to produce male flowers for several to many years before they produce their first female flowers), there is a positive correlation between fruit production by individual trees in different years for all pairs of years, but only 3 out of 6 correlations are significant at 5%. In contrast to the experimental study, there were no significant negative correlations between defoliation and acorn production in any year. This highlights the importance of careful matching of sprayed and control trees before the experimental study began. After two years of pilot study we were able to match our experimental trees for age, growth rate and flushing date. Differences in these parameters cloud the relationship between defoliation and fecundity to such an extent that no significant effect of defoliation on fruit production emerges from the observational study.

2.2. Defoliation

Some trees are consistently more heavily defoliated than others. The correlation between defoliation in one year and defoliation in the next is positive and significant for all pairs of years (Table 1). It is difficult to factor out the effects of microhabitat differences, and impossible to assess the genetic component of this differential herbivore susceptibility, but late-flushing trees are consistently less heavily attacked than early flushing individuals, and large trees consistently more heavily attacked than small ones. Large trees also tend to flush earlier than small ones. These internal correlations all confound the relationship between defoliation and acorn production. We have no way of knowing whether these trends with tree age are genuine ontological shifts, or whether the genotypes of trees planted several hundred years ago were different (e.g. earlier flushing) than those planted more recently.

Table 1. Defoliation of individual trees in different years.

	1982	1983	1984	1985
1982	-	0.583	0.754	0.726
1983	-	-	0.727	0.682
1984	-	-	-	0.740

correlation coefficients based on defoliation scores of 36 individual trees (5% significance value = 0.325).

2.3. Trunk growth

Our preliminary studies were based, like Varley's Wytham Wood study, on only 5 trees. They suggested a positive correlation between growth and defoliation, which we interpreted in the same way as Coley (1983) interpreted the differences she found in growth rates and defoliation levels between different species of trees in tropical forests. She argued that on productive soils, the costs of extra chemical defence exceed the benefits, so plants which put more into growth and less into defence grow more quickly, despite the higher rates of defoliation they suffer. We reasoned that trees with a late flushing date lost more by way of reduced leaf area duration than they gained in terms reduced defoliation. The long term study, however, with its larger numbers of trees, shows the more obvious negative correlation between trunk increment (measured as the increase in log (girth^3)) and defoliation. The partial correlation between defolation and growth remains highly significant even after the confounding effects of tree size and flushing date are taken into account (see above).

2.4. Galling by **Andricus quercuscalicis**

The insect causing 'knopper galls' on the acorns of **Q. robur** has increased rapidly during the past 20 years (Collins et al., 1983), and can destroy over 90% of the acorn crop in certain years (Crawley, 1984). On the 36 trees in the present study, average attack rates varied between a high of 47% in 1983 to a low of 26% in 1984 (Table 2). Neither absolute attack rate (measured either as galls per shoot) nor relative attack rate (percentage of final acorn crop galled) is correlated with other aspects of tree performance, though (like defoliation and acorn production) the same trees are consistently the most heavily galled, year after year. The main factor influencing the rate of galling is proximity to Turkey oak **Quercus cerris** (where the sexual generation forms its galls on the male flowers). Over very short distances, galling rates on English oak can decline from an average of over 4 per acorn cup (400% galling) on trees directly beneath a Turkey oak, to well under 0.1 galls per acorn cup (10% galling) on oaks 100 metres away. There is little in the data to suggest that the rate of galling depends upon the density of the acorn corp, either from tree to tree, or from year to year. Individual trees bearing a single acorn have had that acorn galled, and trees with large acorn crops have suffered almost 100% galling. No evidence has been found of 'predator satiation' (where the largest acorn crops suffer reduced rates of attack), except in 1985, when there was a singificant negative correlation between size of the acorn crop and the percentage of acorns galled.

The population dynamics of the alternating generations of **A. quercus-calicis** have proved rather difficult to fathom. Since the English oak generation (the knopper gall of acorns) becomes visible only 2 months after the emergence of the sexuals from male flower galls in Turkey oak, one might expect a reasonably close relationship between abundance on Turkey oak (male flower abundance x galling rate per male flower) and abundance on English oak (peduncle abundance x galls per peduncle). Indeed, the relationship is so good (Table 2) that we can risk the prediction that knopper galls will be more abundant in the autumn of 1986 than in any year

so far (over 0.2 galls per shoot). In contrast, the relationship between the number of agamic females emerging from knopper galls on the ground beneath English oak in early spring, and attack rates on Turkey oak (again, only 2 months later), is remarkably poor. A combination of heavy losses during dispersal between trees, and severe density-dependent mortality of eggs within the Turkey oak buds, are the most likely causes of the discrepancy (R.S. Hails, in preparation). It is much more likeky, for example, that an individual emerging from a gall on Turkey oak will find an English oak, than that an individual emerging beneath an English oak will find a Turkey oak (given the relative scarcity of the alien Turkey oak). It may be that Turkey oak has been relatively scarce throughout the evolutionary history of the association, since the dispersers of the 'high risk' generation (the agamics with their 1000 eggs) are wastly more fecund than the dispersers of the 'low risk' generation (the sexuals with their 10 eggs).

Table 2. Average reproductive performance in different years

	1982	1983	1984	1985
DEFOLIATION	2.42	2.72	2.86	2.69
ACORNS/SHOOT	0.39	0.21	0.13	0.35
GALLS/SHOOT	0.11	0.12	0.05	0.15
% GALLED	33.0	47.0	26.0	36.0
SEXUAL GALLS	0.92	0.29	0.10	0.57

Means of 36 trees. 1986 sexual galls score = 1.12; predicted 1986 agamic galls/shoot = 0.25

2.5. Tree to tree differences in defoliation dynamics

We began this study with the expectation that there would be high defoliation years and low defoliation years, and that all trees would show comparable increases or decreases in defoliation levels from year to year. Over the 4 years for which we have data, there are 27 possible year to year patterns of change in defoliation, if individual trees are scored as showing '+' (a rise in defoliation score > 0.3 (5% LSD) from one year to the next), '-' (a fall > 0.3) or '0' (no change; increase or decrease < 0.3). For example, a tree may show consistent increase in defoliation (+++), consistent decrease (---), or it may fluctuate in various ways (+0-, ++0, and so on). Far from all showing the same pattern of change (as a simple climatic model might predict), the 36 trees show 17 of the 27 different patterns! There is a modal pattern of change (+-0) over the years 1982 to 1985, but this was exhibited by only 6 out of the 36 trees. The wide scatter of responses highlights the need for very large sample sizes in projects of this kind. Whether these different patterns of defoliation

dynamics are associated with microhabitat factors, or with other attributes of the trees, remains to be seen. For the moment, it is a sobering thought that after 5 years of detailed study we can not predict the direction of change in defoliation levels, let alone the magnitude!

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OVIPOSITIONAL RESPONSES OF THE STEM BORER CHILO PARTELLUS (SWINHOE) TO CERTAIN SORGHUM CULTIVARS IN RELATION TO THEIR RESISTANCE OR SUSCEPTIBILITY

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1. Introduction

The stem borer **Chilo partellus** (Swin.) is a serious pest of sorghum in various parts of Africa and India. However, a number of cultivars of sorghum have been reported to be resistant to the pest (Jotwani et al., 1979; Singh et al., 1982). Among the factors governing the resistance or susceptibility of different cultivars to the stem borer, its ovipositional responses to the plants have been compared by some workers (Lal & Pant, 1980; Singh & Rana, 1984). Their reports are based oin field observations on the number of eggs or egg-masses laid on different cultivars under natural infestation. But, differences observed in the fields in the egg-laying on different cultivars might be due to non-plant factors as well because: (a) the number of insects infesting the plants may vary from time to time and in different parts of the experimental fields; and (b) the stimuli like light intensity and humidity might vary from place to place in the field and might infuence the egg-laying by the insects. In order, therefore, to ascertain the role of different sorghum cultivars themselves in oviposition by the stem borer, the present work has been taken up, employing techniques specially developed for the purpose.

2. Materials and methods

2.1. Test insects and plants.

Newly emerged males and females of **Chilo partellus** were taken from a culture maintained on an artificial diet (Ochieng et al., 1985). The two sexes were allowed to mate for one night and tested for their ovipositional responses on the 2nd night (Kumar & Saxena, 1985). The sorghum cultivars tested were: IS nos 18363, 18463, 2146, 18520, 4660, 2205, 1044. Of these, the first three are susceptible, the 4th one is tolerant and served as the standard reference for comparison with other cultivars, and the last three are resistant to the pest. These plants were grown in the field in a special pattern according to the experiments, as explained below. The age of the plants at the time of the test was 3-5 weeks after emergence, when the oviposition on the crop commences in the field.

2.2. Methods for ovipositional responses to whole plants

These were tested in a 3-sector chamber (210 x 80 x 80 cm), a central sector between 2 equal sized end-sectors on either side (Fig. 1). The chamber's roof and two vertical end-walls were of glass but it was open below, its floor being formed by that of the test arena. The front and rear

walls of the central sector were of glass and those of the end-sectors were of removable wirenet (6 meshes/cm). For each test, the chamber was placed in the field in such a manner that its long axis was at right-angles to the direction of the wind which could then pass through the two end-sectors but not through the central one. 3-5 test plants were arranged inside one end-sector in a row along its end-wall (Fig. 1a). The other end-sector was left vacant in no-choice tests or provided with a similar row of plants of another cultivar in 2-choice tests. Four wax paper strips (15 x 15 cm each) were stuck to the wire-net walls in each end-sector to serve as suitable ovipositional substrates (Kumar & Saxena, 1985) besides the plants. Five females were released in the central sector at dusk for the night. Next morning, the numbers of eggs laid on the plants and the wax paper stips in each sector were recorded. Each experiment was repeated at least 4 times using a total of 20 females or more.

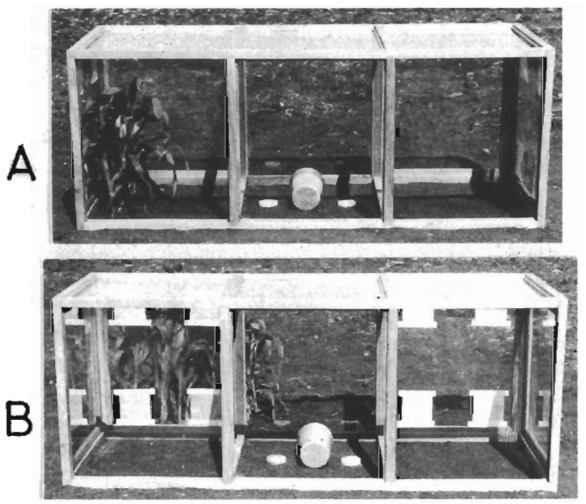

Figure 1. 3-Sector Chamber.

2.3. Methods for ovipositional responses to distance-perceivable stimuli from plants

The method was the same as above except that 12 plants were arranged in 4 rows of 3 each outside the rear wire-net wall of the end-sectors (Fig. 1B). The visual, hygro- (aqueous vapour) and olfactory (non-aqueous volatiles) stimuli from the plants would pass through the corresponding end-sector but not through the central one. The insects hovering to and fro along the length of the chamber could perceive these stimuli in the end-sectors and lay eggs on the wax-paper strips therein without contact with the plants. Differences between the percentages of eggs laid in the two end-sectors would be due to any one or more of the above mentioned distance-stimuli from the plants outside the respective end-sectors each experiment was repeated at least 4 times using a total of 20 females or more.

2.4. Methods for ovipositional responses to contact-perceivable stimuli from plants

For this study, a circular chamber (Fig. 2) was used which consisted of a wire-net base 'b' (11.5 cm dia.; 3.5 cm ht) supporting a removable wire-net cover 'c' (11.5 cm dia.; 1.5 cm ht). A leaf of the test plant was stretched across the chamber between the base and the cover, occupying one half of the circular arena. The other half of the arena was occupied by a non-plant ovipositional substrate, e.g. a glass plate, in no-choice tests or by the leaf of another plant in 2-choice tests. An ovipositing female was released at dusk within the chamber and given a wet cotton wool swab on the cover to drink water from. The insect could move around but remained in contact with one or other of the test materials most of the time. The numbers of eggs laid on the test materials during a night was recorded. Each experiment was repeated with 10 females or more.

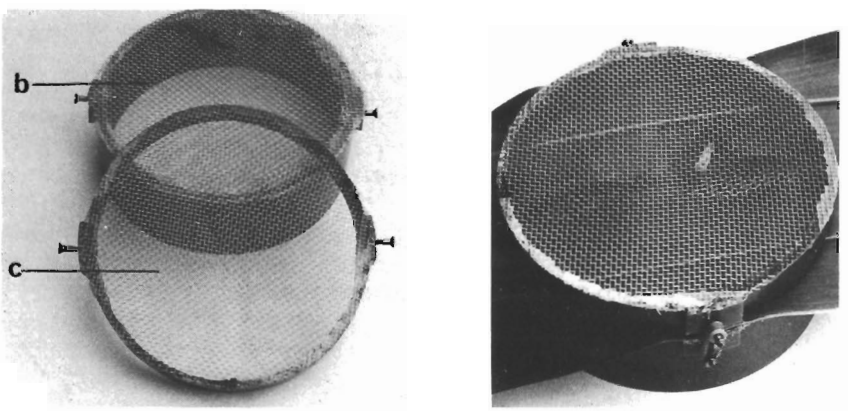

Figure 2. Contact oviposition chamber.

3. Results and discussion

The relative suitability of the test cultivars for oviposition was compared on the basis of: (a) numbers of eggs laid, and (b) ovipositional preference (OP) for the cultivars relative to the non-plant substrate i.e., wax paper serving as the 'blank'. The OP represented the percentage of eggs laid on the plants in excess of that on the blank and was calculated as 100 (A-B)/A+B, A and B being the numbers of eggs laid on the plants and the blank respectively. Positive OP values reflected attraction and/or contact stimulation, negative values avoidance and/or contact inhibition of oviposition by the plants. OP values close to zero reflected lack of involvement of plant stimuli in oviposition.

3.1. Responses to whole plants (Table 1).

The number of eggs laid as well as ovipositional preference were high for not only the susceptible IS nos. 18363 and 18463 but also for the resistant IS 2205. The number of eggs laid was medium but the OP high for the susceptible IS-2146 as well as the resistant IS-4660. For the tolerant

IS-18520, the number of eggs laid as well as OP were medium whereas the resistant IS-1044, for which the number of eggs was low and OP medium, was least suitable for oviposition.

3.2. Role of distance stimuli (Table 2).

The distance-stimuli from the plants, presented outside the wire-net wall of the chamber, were most effective in eliciting ovipositional response to the susceptible IS nos. 18636 and 2146, less effective for the susceptible IS-18463 and least effective for the tolerant IS-2205 and IS-1044. The distance-stimuli involved in the above differences in oviposition on different cultivars would be olfactory since the visual and hygro stimuli from these plants have been previously shown no to be involved.

Table 1. Ovipositional responses of **Chilo partellus** to certain sorghum cultivars, each presented alone inside one end-sector of the 3-sector test chamber in the field.

Test cultivars (IS nos.)	No. females tested	No. eggs/5 females		Ovipositional preference for plants (mean ± s.e)
		Plants (A)	Blank (B)	
18520 (Serena)	45	137.8 ± 29.1	28.1 ± 8.4	62.1 ± 14.4
18363	40	177.1 ± 43.1	12.3 ± 5.9	77.9 ± 11.4
18463	55	178.5 ± 52.9	15.6 ± 9.9	76.2 ± 13.8
2146	55	94.5 ± 17.7	3.2 ± 2.6	97.8 ± 19.5
4660	40	108.8 ± 38.6	13.9 ± 9.2	94.1 ± 4.6
2205	55	189.2 ± 35.0	13.9 ± 8.9	95.6 ± 2.9
1044	50	57.9 ± 14.2	16.6 ± 6.5	61.1 ± 19.5

3.3. Responses to contact-stimuli (Table 3).

On contact with the leaves of the test cultivar offered as a choice against the tolerant check IS-18520, the susceptible IS-2146 was more effective and the resistant IS-1044 less effective in eliciting oviposition. The remaining cultivars were almost as effective as IS-18520. But, an additional cultivar IS-23175, which has very hairy leaves, inhibited egg-laying. In fact, the hairy leaves were worse than even IS-1044 in receiving oviposition. Such a low oviposition on IS-23175 would be because of their hair which, when removed, received a higher number of eggs.

Thus, ovipositional non-preference caused by a lack of adequate olfactory stimuli and presence of hairs on certain sorghum cultivars e.g., IS-1044, IS-23175, respectively, contributes to their resistance to **C. partellus** but is not involved in the resistance of IS-2205 and IS-4660.

Table 2. Ovipositional responses of **Chilo partellus** to distance-stimuli from different sorghum cultivars, each presented alone outside the wire-net wall of one end-sector of the 3-sector test chamber in the field.

Test cultivar (IS nos.)	No. females tested	No. eggs/5 females (mean ± s.e)		% ovipositional preference for plants (mean ± s.e)
		Plants (A)	Blank (B)	
18520	20	125.0 ± 54.1	37.0 ± 16.2	33.0 ± 28.1
18363	20	66.7 ± 14.9	22.3 ± 19.9	70.6 ± 29.4
18463	20	116.0 ± 15.5	57.0 ± 14.2	34.8 ± 14.3
2146	20	49.7 ± 11.8	1.0 ± 1.0	94.3 ± 5.7
4660	20	83.7 ± 10.8	28.7 ± 18.3	56.2 ± 19.6
2205	20	72.0 ± 27.8	40.7 ± 13.1	26.5 ± 2.9
1044	20	45.0 ± 27.3	15.8 ± 3.5	20.6 ± 30.4

Table 3. Ovipositional responses of **Chilo partellus** to contact-stimuli from different sorghum cultivars (2-choice situation).

Test cultivars (IS nos.)		No. females tested	No. eggs/5 females (mean ± s.e)		Ovipositional preference for A
A	B		A	B	
18463	18520	29	524.7 ± 160.8	559.3 ± 129.5	-4.1 ± 18.2
18363	18520	42	495.8 ± 116.9	398.5 ± 43.3	7.0 ± 9.5
2146	18520	65	544.8 ± 52.4	234.3 ± 16.0	39 ± 3.6
4660	18520	32	275.3 ± 52.8	319.7 ± 126.6	-2.5 ± 13.7
2205	18520	36	294.4 ± 70.4	318.8 ± 60.1	-6.8 ± 10.5
1044	18520	114	252.2 ± 55.5	435.8 ± 92.6	-26.4 ± 8.1
23175 (Hairy)	18520	10	3.0 ± 3.0	117.3 ± 40.6	-97.8 ± 2.2
23175 (Hairy)	1044	10	1.1 ± 1.1	126.9 ± 29.5	-97.7 ± 2.3
23175 (Hairy)	23175 (Hair removed)	13	37.6 ± 12.9	93.3 ± 19.2	-45.3 ± 15.2
23175 (Hairy)	Glass	10	25.7 ± 8.3	29.9 ± 12.4	-13.0 ± 27.5

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CHAPTER 7. COEVOLUTION AND COSPECIATION MECHANISMS BETWEEN PLANTS AND INSECTS.

ON THE ROLE OF VOLATILE CHEMICAL SIGNALS IN THE EVOLUTION AND SPECIATION OF PLANTS AND INSECTS: WHY DO FLOWERS SMELL AND WHY DO THEY SMELL DIFFERENTLY?

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1. Introduction

Why do flowering plants smell? Why do different species smell differently? What role does volatile compounds have for the pollination by insects? What is their ecological meaning? How have chemical signals between plants and insects evolved? - There are some of the questions we want to address by performing chemical analyses of flower volatiles and behavioral observations and experiments of pollinating insects as well as morphological and ecological studies.

Comparatively little is yet known about the chemistry of fragrances from flowering plants. Of course, perfumery chemists and natural product chemists have laid a foundation for this knowledge, but much remains to be done. Especially, comparative studies based on thorough analyses of plant volatiles from taxonomically related species or plants that grow together are needed. Such work should be correlated as much as possible with morphological, ethological and ecological observations and experiments. Studies of various kinds of variation in the production and giving off of fragrances, for example daily and seasonal variation and variation between individuals and populations, ought to be done.

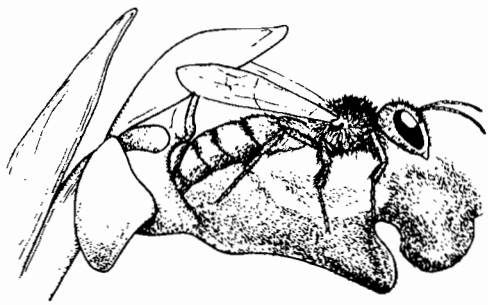
The examples in the present contribution is taken from our collaborative work on orchids and some other plants and their pollinators. It represents cooperations between biologists and chemists and emanates from the studies started in the early 1960-ies by B. Kullenberg (Uppsala) and E. and S. Stenhagen (Gothenburg) together with their Swedish and international colleagues.

The interdependance between flowering plants and insects and their mutual evolution has been well formulated by the French botanist F. Moreau (1955), cited by B. Kullenberg (1984): "C'est l'insecte, l'insecte butineur, qui a fait la fleur - la fleur entomophile - au cours des temps tertiaires et, dans le même temps, c'est la fleur - tout au moins la fleur entomophile - qui a fait l'insecte butineur. Ainsi s'affirme au cours des âges la solidarité du monde des plantes et de celui des insectes".

2. Pollination of ophrys orchids by aculeate Hymenopteran males

Ophrys orchids -. there are more than 20 species/forms around the Mediterranean and Southern Europe - are pollinated solely by aculeate Hymenoptera males, which do not receive any nectar from the flowers. Instead they are lured to the plant by sexual stimuli and "pseudocopulate" with the flower labellum (see Kullenberg, 1952, 1961, 1973 and Kullenberg &

Bergström, 1976a and b). Both olfactory, visual and tactile cues are responsible for this attraction/excitation of the pollinators.



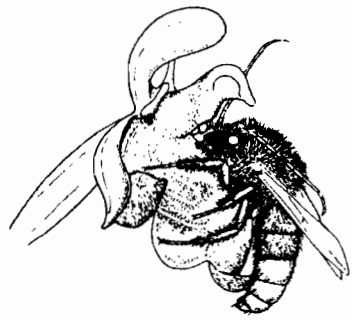
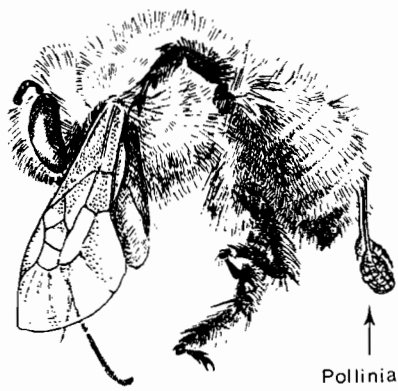


Figure 1. (left) Pollination of **Ophrys fusca** by a male **Andrena**. The pollinia are taken by the tip of the abdomen of the insect. Figure 2. (right) Pollination of **Ophrys scolopax** by a male **Eucera**. The pollinia are taken by the head of the insect.



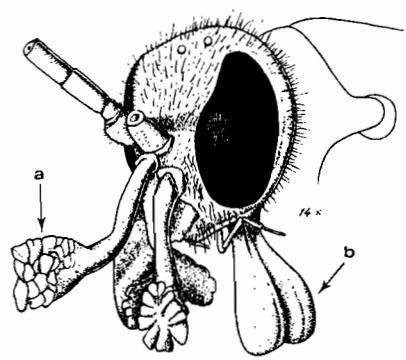


Figure 3. (left) **Anthophora acervorum** male with pollinia on the tip of the abdomen visiting **Ophrys fusca**. Figure 4. (right) **Argogorytes fargei** (syn. **campestris**) head with pollinia of a) **Ophrys insectifera** and b) **Listera ovata**.

We have studied the chemosphere around **Ophrys** orchids of many different species and forms (see for example Kullenberg & Bergström, 1976a and b; Borg-Karlson et al., 1985) and found that it is made up by a complex mixture of compounds with varying polarities and chemical structures. Fatty acid derivatives, isoprenoids (mono- and sesquiterpenes) and benzenoid compounds form these blends that have been found to be specific for each species and form.

The relationship between **Ophrys** and its pollinators represent one case of chemical mimetism in the sense that several compounds have been found that are present both in the **Ophrys** species and in the pollinating

insects. Behavioral experiments have been performed (see for example Kullenberg op. cit. and Borg-Karlson, 1985), which show that some of the compounds are indeed guiding the insect. Each species/form **Ophrys** has only one or just a few major pollinating species, usually solitary bees or sphecid wasps.

The interplay with plants may be one raison d'être for the complexity of volatile signals, especially in Hymenopteran insects. With a blend of compounds of different volatility, there exists the possibility of gradients - both in time and space. **Ophrys** pollinators may be attracted from long distances by certain compounds - we suspect the sesquiterpenes, while other ones - fatty acid derivatives, monoterpenes and benzenoid compounds - are responsible for the excitation within visual and tactile contact. We believe that there might have been a "proto-**Ophrys**", which produced nectar and was pollinated by both males and females. Maybe, then, there was a small difference in the behavior of the males and the females on the labellum - maybe the males were more excited or stayed a bit longer - which was reinforced by the evolution of the flower so that concurrent with diminishing nectar production males became the exclusive visitors (see further Bergström, 1978, 1979).

3. Analytical techniques

To collect small amounts of often complex mixtures of natural volatile blends we have been using several different methods for different applications. The four most current techniques are: sorption, cold trap collection, pre-column evaporation and solvent extraction. Of these, the first two are accumulating methods, which enrich volatiles over time, whereas the two latter ones are cross sectional methods which collect the volatile material available at a certain time. To obtain a possibly true picture of the proportions of the components of the natural blend, we recommend that one combines one accumulating and one cross sectional method, for instance: sorption plus solvent extraction. For further discussion of this, see Bergström, 1980.

The collected volatile material has normally been subjected to capillary gas chromatography and mass spectrometry. Different microchemical techniques, such as hydrogenation and ozonolysis have often been applied. To collect small, sharp fractions a revolving fraction collector has been used (Wassgren & Bergström, 1984a and b). The collected fractions are used either for behavioral experiments or for further chemical analysis.

4. Floral fragrance disparity between three species of Cypripedium

Using the methods described, volatiles have been analysed and compared from three species of **Cypripedium** (Orchidaceae), viz. **C. calceolus, C. parviflorum** and **C. pubescens**. It turns out that the perfume given off by these species are drastically different from the chemical point (Bergström, Nilsson et al., in press). Each species is characterized by substances belonging of one class of chemical compounds. The major volatiles from **C. calceolus** are a series of straight chain acetates. The fragrance of **C. parviflorum** is dominated by terpenes, the largest components being cis- and trans- β-ocimenes. In **C. pubescens** there is one major component. This was

identified as 1, 3, 5-trimethoxy benzene. Behavioral tests will now be performed to see what these compounds mean in the attraction/excitation of pollinators.

5. Comparative analysis of floral odors from Actaea spicata **and** A. erythrocarpa

Analyses of volatiles form several species of **Actaea** (Ranunculaceae) have been made by Pellmyr, Groth and Bergström (1983 and in press). These studies are also aimed at understanding the relationships between the plants and their pollinators, which are beetles (Coleoptera; Byturidae). It was found that the **Actaea** are veritable producers of monoterpenes (see Figures. 5 and 6). All the major components in the complex secretion are simple monoterpenes, which are very common in plant odors, such as: myrcene, gernanylacetate, nerol and geraniol. There are considerable similarities in the setup of volatiles in the five species analysed, but there are also characteristic differences. It has been suggested by Faegri and van der Pijl (1979) that the fruit odors among primitive angiosperms were evolved to mimic actual fruit odors and it may well be that odor preceded visual cues as an effective attractant for pollinators.

In the second work we used a technique whereby we could separate out the volatiles actually produced by the flowers. Parallel collections were made from inflorescence and vegetative parts.

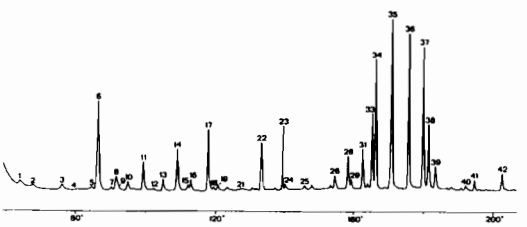


Figure 5. Typical capillary gas chromatogram of the floral odor form **Actaea spicata.**

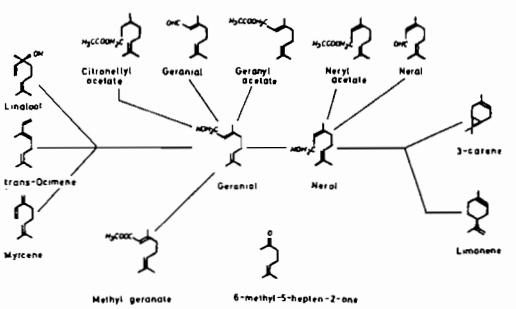


Figure 6. Chemical structures of major monoterpenes in the floral odor of **Actaea spicata.**

6. Volatile signals in the pollination of Zygogynum **and** Exospermum **(Winteraceae).**

In New Caledonia there are primitive and vesselless angiosperms of the genera **Zygogynum** and **Exospermum** that belong to the family Winteraceae. They are pollinated by a likewise primitive moth, **Sabatinca** (Micropterigidae). The insects use the flowers as mating sites and eat the pollen. We have analysed the odors given off from the flowers (Thien et al., 1985) and we have found that they are composed mainly of species specific blends of fatty acid derivatives and isoprenoids. The latter group of compounds include mono- and sesquiterpenes as well as some irregular isoprenoids with 13 carbon atoms. The compounds span a wide range of volatilities. Ethyl acetate, which is present in the secretions in large amounts, may well be a nauseating agent that keeps the pollinator in the flower for a longer time. Thus increasing the pollination capability. The mode of pollination in which flowers serve as mating and feeding stations with floral odors acting as cues may have been common in the early evolution of flowering plants.

7. Some comments about volatile chemical signals in the evolution and speciation of plants and insects, with special reference to pollination.

Already primitive unicellular organisms must have had the capacity - by chemotaxies - to move up to nutritional sources and away form "enemies" and toxic material. Early on, then, there was probably a division between attraction and repulsion; maybe these separate types of behavior were elicited by different chemical signals. In the relationship between plants and insects, we can see the same dualism: plants need insects for pollination and seed dispersal and must avoid being eaten; insects need plants for food, mating stations and nesting, but must avoid being exposed for toxic compounds from them. This mutualism in the biocoenosis has evolved sets of adaptation on different levels.

Biochemically, there are considerable similarities between plants and insects, particularly in their production of volatile compounds. Fatty acid derivatives, isoprenoids, benzenoid substances and compounds containing heteroatoms - especially N and S - are examples of classes of compounds, which are produced and excreted or secreted by both groups of organisms. This reflects similarities - and adaptations - on the protein level, in biosynthesis and in chemoreception.

In our studies on volatile compounds from bees, especially substances emanating from the mandibular gland, we have found that a great many fatty acid derivatives and isoprenoids are produced (Tengö & Bergström, 1976, 1977; Bergström & Tengö, 1978; Bergström et al., 1982). Esters are especially common in the volatile secretions of bees (Francke et al., 1984). Many of these compounds from pairs that occur both in the plants and in the insects. This is the case for **Ophrys** and pollinating bees, and it is also true for **Cypripedium** and their bee visitors. In the case of **Actaea** and the Winteraceae plants, referred to above, we do not yet know anything about the volatile compounds from the pollinators.

Most volatile excretions/secretions involved in plant/pollinator relationships have been found to be very complex. Certainly, not all of the compounds are behaviorally active. What is the raison d'être for all these

substances? Some are very likely artefacts, biochemical precursors or excretion products. Still, there are several compounds which are active and by combination they can serve many purposes. One is that gradients - both in time and space - can be generated by mixtures of compounds with different volatilities. Another is that complexity is one way to achieve specificity. Complex secretions can also form a basis for adaptation between plant and insect, either by innate mechanisms or by learning.

There seems to be a strong connection between the vital needs of food and mating on the insects part. The plants seem to find ways to manipulate these drives for their pollination by employing mimics of the insects chemical signals. In some cases they are downright food cues. In other cases, exemplified by **Cypripedium**, which act by deceit, no nectar is produced by the odors and other signals direct the pollinator to the plants until the false message has been learnt. In the very special case of **Ophrys**, where the pollination is highly assortative and performed only by sexually excited male bees, the plant mimics insect volatiles which might well earlier have been food signals but which are now part of the species recognition signal.

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THE INFLUENCE OF THE MICROENVIRONMENT -THE INTERIOR OF THE SYCONIUM- IN THE COEVOLUTION BETWEEN FIG WASPS (AGAONIDAE) AND THE FIG (FICUS)

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1. Introduction

The development of seeds in **Ficus** depends upon small wasps of the family Agaonidae which cannot develop anywhere except in the gall flowers. Each species of fig has a specific pollinator with few known exceptions and related groups of figs (taxa) have related pollinators (Ramirez, 1970, 1974, 1977; Wiebes, 1982a, 1982b). In fig symbiosis coordination of development of the participating organisms (the syconia and the agaonid larvae) is of vital importance. For the maintenance of the Agaonidae-**Ficus** association and the survival of both participants, extreme flexibility of the adapting mechanisms is indipensable. The success of the symbiosis can be ensured by the imposition of the devopmental rhythm on the most sensitive component: the wasp (Galil & Elsikowitch, 1971).

The closed dark and humid microenvironment of the syconial cavity and its particular gaseous concentration have produced small isolated habitats that have brought about morphological, behavioral and ecological traits in the Agaonidae that make them fit into the Local Mate Competition Rule of Hamilton (1967). One of the specifications of this rule is that there be few females colonizing the host, which leads to a close inbreeding system.

2. Agaonidae and the local mate competition rule (LMC)

In Agaonidae the males are a product of arrhenotoky; a mode of reproduction that enhances the production of biased sex ratios (Hamilton, 1967). According to Hamilton, it seems that male haploid organisms are preadapted for life in isolated habitats or hosts of the sort characterized by his model. The evolution of male haploidy has actually accompanied an evolutionary trend to occupy such habitats in several independent lines. Most of the arthropods listed by Hamilton (1967), which develop in isolated hosts and which have both arrhenotoky and spanandry, are hymenopterous insects (including Agaonidae) and a few mites (Acari). Green et al. (1982) suggested that "an evolutionary advantage of arrhenotoky is that it makes possible precise sex ratios, which provide a selective advantage in highly inbred parasitic wasps".

In the Agaonidae the males are wingless, smaller, neotenic, unpigmented, with reduced eyes and no ocelli. The evolution of these characteristics can be attributed to their development in a dark and humid environment, namely the interior of the syconium.

The high relative humidity and carbon dioxide concentration inside the developing syconia are probably higher than those in the environment for

most species of **Ficus** when the male adult agaonids are emerging from the galls. Galil et al. (1973) found that during the early stage of the male phase of **Ficus religiosa** the internal atmosphere of the fig consisted of about 10% Co_2, 10% O_2 and some ethylene. The males of the pollinator **Blastophaga quadraticeps** carry out their usual activities in perforating the walls of the galls and fertilizing the females under these conditions. After perforation of the syconial wall by the males, the gas composition of the syconium becomes equilibrated with the external atmosphere. As a result the male wasps are inhibited, whereas the females become active, leave the galls, load their pollen pockets and emerge from the syconium. The author found that the pollinators (**Pegoscapus**) of the New World **Urostigma** behave as **B. quadraticeps** in relation to the concentration of CO_2 and O_2 when emerge from their galls and syconia at male phase.

According to Galil et al. (1973) "A CO_2 enriched atmosphere is known to play an important role in the lives of various parasites and underground animals in habitats where there is an increased CO_2 percentage as a result of the metabolism of the animals". The dark microenvironments, gaseous conditions and high relative humidity in the syconium have led to radiative adaptation, synchronization of development for at least one of the associates, and total dependence for both partners.

The Agaonidae have developed a rather close inbreeding system, and copulation occurs right after emergence of the males from the galls inside the host (the syconium). According to Mitchell (1973) "all adaptations for small territories tend to result in inbreeding ". In the Agaonidae the developmental period is very constant and each syconium is colonized by a small number of females (Fig. 1) and the galls are eventually exhausted through feeding of the progeny.

To the test how many females and in what distribution they colonized the syconia, as well as the sex ratios, the following analyses were performed. A sample of 25 syconia per branch, from eight branches of **Ficus citrifolia** was used to count the number of female pollinating wasps that penetrated the syconia. Due to the skewness of the frequency distribution to the left and the type of variable, a Poisson distribution was hypothesized. The goodness of fit between the frequency of occurrence of the observations in the sample and the expected frequencies obtained from the hypothesized distribution, was tested using "Chi-square criterium" (Steel & Torrie, 1980). The test showed that there was good fit between the observed and expected values ($X= 8.94$, $\alpha > 0.05$). The penetrating females of **B. estherae** follow a Poisson distribution when they colonize the syconia of its host (**F. citrifolia**). With the frequencies as dependent variables and the values of the random variable (number of wasps per syconium) as independent variable, a regression analysis was performed in order to establish the relationship between the variables. The closest agreement was found by using a Gamma model ($R^2 = 98.8\%$). This model has a sound first derivative and therefore a maximum, which in this case was found to be 1.7 (Fig. 1). An analysis of variance showed no differences in the number of penetrating females between branches ($1\alpha = > 0.05$) and that the sample was homogeneous. Fifty syconia (at D phase) selected at random in three strata of a tree were collected. It was found that there was no difference

($P<0.05$) between strata nor between branches orientated differentely in relation to the number of adult agaonids and seeds produced per syconium. **F. citrifolia** sacrifices one third of the female flowers for the survival of its pollinator. The syconia of six other New World species of **Urostigma** and **Pharmacosycea** were found to be colonized by a few females. Most of them had less than 3 (maximum 7).

A correlation analysis showed that there was a good linear relationship between female and male counts ($r^2 = 0.6364$) and between counts of seed and total wasps ($r^2 = 0.3230$); however the latter was not as strong as the former.

When the numer of eclosing female wasps per syconium was considered as the independent variable and number of males as the dependent variable, a regression analysis showed that the best fit was found to be a potential model that yielded a ratio of one male per 37 females (Fig. 2). The sex ratios reported for other sycophilous wasps are: for **B. psenes**, 0.9 to 0.18 Joseph (1958), **Sycophaga sycomori** 0.36, **B. quadraticeps** from 0.2 to 0.78, mean 0.40, Galil and Eisikowitch (1971), **Ceratosolen fusciceps** 0.39, Joseph (1984).

From the data available about the number of colonizing agaonid females per syconium at B phase in different species of **Ficus** (belonging to different taxa) and different groups of agaonids, one can conclude that only a few females (1-2) are enough to pollinate a given syconium, Cunninham (1889), Baker (1913), Kjellberg et al. (1983), Michaloud and Michaloud-Pelletier (1983).

Due to the low number of colonizing wasps per syconium, at female phase*, (usually one or two), the Agaonidae have developed a rather close inbreeding system. Inbreeding among LMC organisms does not seem to be a problem since a high genetic variation is not necessary, because they spend most of their lives (development, sexual maturation and oviposition) in very constant physical and alimentary environments. Among the characteristics of Agaonidae that seem to fit into Halmilton's rule are: specific relationships between host and associate; females usually lay a constant number of eggs; nearly constant developmental time from egg to adult of the associate; neotenic and smaller males; very constant ontogenies of one of the associate; spanandrous sex ratios; gregarious development; few females colonizing the host; quite constant number of progenies reared per host and time; males born before the females; shorter male life span; no feeding of males; mating occurs before females emergence from the galls; polygamous males; sibmating more common that outcrossing; mating in same place of development; no males successfully mate outside their own group; no abandoning of males of place of development (claustrophobic males); females able to store sperm; total egg production fertilized by one insemination. Other characteristics of Agaonidae which probably agree with the Low Mating Competition Rule could be: modification of the developmental period and morphology of the host; developmental synchronization of host and associate, fighting of females at oviposition and of males at mating time.

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* For developmental phases of the syconium, see Galil & Eisikowitch (1968).

3. Morphological adaptations of the Agaonidae in relation to the internal environment of the syconium

Evolution in females of ovipositors as long as or longer than the length of the styles of the gall flowers, development of topocentric pollination by those wasps inhabiting fig species in which the pollen comes out naturally from the anthers and evolution of pollen pockets and/or coxal corbiculae to carry pollen in those fig species in which it does not come out naturally from the anthers (ethodynamic pollination). The males have long tubular gasters (solenogastry) to copulate with females while they are inside the galls, reduced antennae with apical sensilla and very strong fore and hind legs.

4. Discussion

In the fig-agaonid symbiosis, extreme flexibility of the adapting mechanisms and synchronization of development are indispendable for ensuring their survival. The success of the symbiosis has become ensured by the imposition of the developmental rhythm of the most sensitive component: the Agaonidae. The fig syconium is an isolated habitat and the Agaonidae have developed many behavioral, morphological and ecological characteristics that fit into the Local Mate Competition Rule (LMC) of Hamilton (1967). The similar microenvironments, gaseous conditions and high relative humidity inside of the syconium seem to have led to radiative adaptations, synchronization of development, and total interdependence for both partners.

The Agaonidae males reproduce by arrhenotoky, which readily permits the production of biased sex ratios (Hamilton, 1967). The evolution of haploidy has actually accompanied an evolutionary trend to occupy isolated habitats or host. Arrhenotoky leads to precise sex ratios which provide an evolutionary advantage in highly inbred parasitic wasps (Green et al., 1982). Due to the low number of colonizing wasps per syconium (usually one or two), the Agaonidae have developed a rather close-in-breeding system.

The colonizing agaonid females seem to follow a Poisson distribution. This pattern shows that there is a high probability of finding one or two colonizing wasps per syconium and that for most species of **Ficus** this number suffices to pollinate all the flowers of the syconium (see Cunninham, 1889). The analysis of variance of the number of females penetrating the syconia in several strata of a tree of **F. citrifolia**, showed that there is no difference between strata; therefore, it is presumable that the colonization is not determined by micro or macroenvironmental conditions of the tree. Using a Gamma model, a maximum of 1.7 penetrating females per syconium was found.

A correlation analysis showed that there was a good linear relationship between females and males counts and between counts of seeds and total wasps; however, the latter was not as strong as the former. A regression analysis yielded a ratio of one male per 37 females for **Blastophaga estherae**. In LMC organisms a high genetic variation does not seem necessary since they develop in very constant environments. Most species of **Ficus** approximately sacrifice from one third to half of the female flowers per syconium for the survival of Agaonidae.

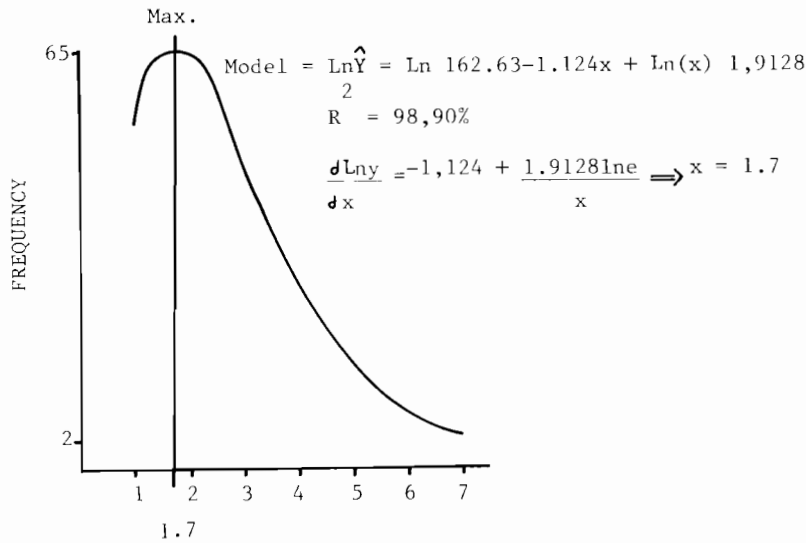

Fig.1 Frequency distribution of the pollinating females within the syconia

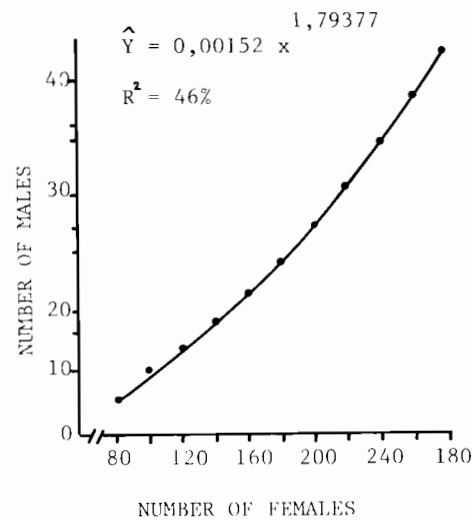

Fig.2 Regression analysis showing unbalanced sex-ratio of the eclosing generation of the pollinating fig wasps

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THE FICUS FICUS-POLLINATOR MUTUALISM: HOW CAN IT BE EVOLUTIONARY STABLE?

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1. Introduction

Although much work has been performed on the **Ficus Ficus**-pollinator mutualism the system has seldom been treated by people trained in population genetics. The aim of this paper is to demonstrate that population genetics can provide a new insight into an old problem.

2. The system

The genus **Ficus** has a unique pollination system. Each species has its own symbiotic pollinator species of wasp that breeds in the female flowers. The inflorescence is located within a closed urn-shaped receptacle called a syconium (sukos is the Greek word for the Latin fica). The inside of the syconium is lined with one to several hundred uniovulate female flowers and some male ones. The mated female wasps carry pollen and enter the syconium when the female flowers are receptive. They then pollinate the flowers and in some of them lay en egg. The ovary develops either into a seed or into a gall containing the agaonid larva. As adults the wasps mate within the syconium and then the females leave it searching for a receptive one in which to oviposit. Most pollinator species are active, that is the females load pollen with a special behaviour within the syconium from which they are emerging and then unload it in the receptive syconium. Some species do not have the active behaviour: they become passively pollen-loaded while emerging from the syconium. **Ficus** species are either "monoecious" or "dioecious". In the monoecious species all syconia produce wasps and seeds. In the dioecious species, the syconia of the "female" trees do not contain any female flower fit for oviposition, therefore they produce no pollen vectors nor in fact do they produce any pollen. The syconia of the "male" trees contain many female flowers, but these produce only wasps and no seeds; as the trees produce pollen, they are functionally male.

3. The evolutionary problem

Both the wasp and the fig benefit from the mutualistic interactions as none of them could reproduce without the other. In a teleological approach this seems to explain why the system is stable and allows the existence of some 700 **Ficus** species. It should be noted however that the generation times of the wasp and the tree are very different as the first should be counted in months and the second in decades. Therefore a female wasp draws no direct benefit for her offspring from ensuring the reproduction of the

tree. One may therefore wonder why short term selective pressures on the wasps do not lead them to stop being pollinators, leading eventually to the disappearance of both the tree and the wasp. The basic points on which short term selection could work are:

- **Why not oviposit in all female flowers?**
- **Why pollinate actively?**
- **Why not avoid female trees in dioecious species?** (this point will not be treated in this paper).

These considerations are not merely theoretical speculations. Field observations on **Ficus sycomorus** (Galil & Eisikowitch, 1969) show that a pollinator can become a parasite: **Ceratosolen galili** is closely related to the pollinator **C. arabicus**, it has pollen pockets to carry pollen but it has lost the pollen loading and unloading behaviour. In Israel, where **F. sycomorus** is a planted tree, only **C. galili** occurs and not **C. arabicus**, so that there is no seed production. In eastern Africa, both **Ceratosolen** species coexist competing within the syconia for oviposition sites. If **C. galili** managed to supplant **C. arabicus**, reproduction of **F. sycomorus** would cease and within some hundred years the tree would disappear together with the wasp. These basic questions about the stability of the symbiosis have seldom been addressed in an evolutionary context.

4. Flower protection against oviposition

4.1. The style length hypothesis

In almost all papers a simple explanation of why the wasps do not oviposit in all female flowers is accepted. The wasps oviposit by inserting their ovipositor through the stylar canal and only deposit an egg if it reaches the bottom of the canal. Female flowers would have either styles that are short enough to enable the pollinator to reach the ovary or too long. The length of the style would then be the means by which the tree balances and controls wasp infestation and seed production.

4.1.1. Evidence on the style length hypothesis. Some evidence supports this explanation. In the dioecious **Ficus carica**, there are two different kinds of syconia according to the time of the year (Kjellberg et al., in press): those for which the apparent buds over-winter on the tree (we will call these delayed syconia) and those for which the buds develop into syconia during a single year (which we will call undelayed syconia). In this dioecious species the pollination cycle is such that delayed syconia cannot produce seeds due to lack of pollination while undelayed ones can. Quite logically female trees produce almost no delayed syconia. It has been observed that within each sex, delayed syconia (that are only a means of producing pollinators on male trees and are useless on female trees) have shorter styles than undelayed ones. This observation can be explained by admitting that there is a correlation between style lengths in male and female trees for each crop. For the undelayed syconia, selection on female trees for long styles protecting against oviposition leads to longer styles even on male trees while for delayed syconia selection acting only on male trees to ensure proper oviposition leads to shorter styles. Two odd unpublished observations support this interpretation. Valdeyron observed a

female tree on which some oviposition had occurred: it was in delayed syconia (relatively short styled flowers). Meanwhile Raymond (personal communication) observed a male tree on which the wasps did not manage to oviposit: it was in undelayed syconia (relatively long styled).

For monoecious figs, the only rigorous evidence is given by Galil and Eisikowitch (1971). They observed on **Ficus religiosa** that in autumn, smaller than average syconia are produced which have below average style lengths. Some such syconia produce only wasps and no seeds.

However a major shortcoming of evidence on the style length hypothesis is that, apparently, all published ovipositor lengths are in fact measures of the sheath of the ovipositor (Wiebes & Eisikowitch, personal communications) and therefore give a rather rough underestimate. Indeed there are almost no measures of style lengths available. Bronstein (in prep.) has shown that in **F. pertusa** many flowers that have style lengths compatible with oviposition are not used by the pollinator even when oviposition was not limited by egg availability.

4.1.2. Style lengths and theory. From an evolutionary point of view, style lengths alone can hardly be accepted as the ultimate reason why some flowers are protected against oviposition and therefore produce no seeds: there should be a constant evolutionary race between the wasp and the fig. The wasp would permanently be selected for longer ovipositors and in response the fig for longer styles. There seems to be no inherent difficulty in selecting longer ovipositors in fig pollinators as their closest allies are parasites of the system, many of them having very long ovipositors as they deposit the eggs into the ovaries from the outside of the syconium. The selection for longer ovipositors should be particularly efficient in monoecious figs for which the distribution of style lengths is continuous and seldom, if ever, bimodal (Bronstein in prep.; Michaloud, unpublished results; Galil & Eisikowitch, 1968). With such style length distributions any increase in ovipositor length will give access to more ovaries and therefore be selected for. The relatively short ovipositors of fig pollinators do no indicate any such selection. The absence of an evolutionary race favouring longer styles is further sustained by the observation (Corner, 1978) that very long styles although possible in **Ficus** are only documented in the female syconia of two dioecious species **F. macrostyla** and **F. squamosa** (12-18 mm and 6-10 mm respectively against 0.5-2.5 mm in the other dioecious species and 2-5 mm as extremes in monoecious **Ficus**).

4.2. A possible function of style length differentiation

If we admit that, in monoecious figs, different style lengths are not responsible for the allocation of flowers to galls and seeds, we can propose an alternative explanation of their presence. One may expect that in **Ficus**, as in many other species having many flowers grouped on a receptacle, all stigmata should reach approximately the same level so that none is hidden from pollen by the neighbouring flowers. This is achieved among composites by having flowers of identical size. If all female flowers were identical in **Ficus** the number of flowers that could be packed within a

syconium would be proportional to the square of the radius while the volume is proportional to the cube of the radius. So in species with large syconia much of the volume would remain unused. One way of using space more efficiently is to pack the flowers in several layers. Flowers with different pedicel lengths are then produced. The differences in pedicel lengths are compensated by complementary style lengths so that a regular stigmatic surface is obtained.

Three observations sustain the flower packing hypothesis. Firstly, after pollination and or oviposition in syconium, the ovaries increase in diameter while there is a differential pedicel growth so that the whole cavity of the syconium becomes crowded with the ovaries (Cunningham, 1889); these ovaries would not have had enough space to develop were it not for pedicel growth. Secondly, in syconia of **Ficus damaropsis**, the largest of all syconia according to Corner (1978), "the flowers are borne on lobing processes from the inner surface, which is unlike the even interior of the majority of figs". Such processes do also occur in the large syconia of some other groups of **Ficus**. This observation can be explained by an optimal space use hypothesis. Several layers of flowers enable **Ficus** to have more flowers since pedicels are less thick than ovaries. There is however a limit to this process as the thickness of a pedicel cannot be negleted. The larger the number of layers, the fewer ovaries can be located in the lower layers as all the pedicels from the above layers are present. Hence, when the internal cavity of the syconium is large, lobing processes that increase the internal surface are necessary in order to increase the number of flowers packed into the syconium. Thirdly, in a scanning microscope picture, shown to us by Verkerke, of a male syconium of the dioecious **F. asperifolia** two layers of flowers having different style lengths compensating for different pedicel lengths were clearly apparent. This demonstrates that style length differences are not obligatorily linked with protection against oviposition.

4.3. Why not oviposit in all flowers?

4.3.1. In monoecious figs. There is no unique and entirely satisfactory explanation of why female wasps do not oviposit in all female flowers. The possibility we favour is that flowers are not only differentiated according to style lengths but also stigmata and the thickness of the style are different, so it may be rather difficult to oviposit in the long styled flowers even if ovipositor length is sufficient.

4.3.2. In dioecious figs, experimentation can be performed on **Ficus carica:** we can mimic experimentally what would happen if all the wasps had ovipositors long enough to oviposit in all the flowers of the female trees. Syconia on male trees can set seed but usually they only produce very few as they are not receptive when functional pollen is produced i.e. when female trees are receptive. In 1985, exceptional frost induced an early receptivity of syconia on male trees, at the same time as on female trees when plenty of pollen was available. These syconia were visited by the wasps and oviposition and pollination occurred. Within most of them several hundred wasp larvae developed as well as many seeds (over 50). Many such

syconia ripened in autumn becoming sweet like female syconia while the larvae had not yet metamorphosed into adults. Despite the heavy oviposition, no wasps emerged from many such syconia. Apparently, the seeds, when sufficiently numerous, made the syconia ripen too early for the pollinator. These results were obtained with several wasps colonizing each syconium. With rare long ovipositored wasps we can assume that only one female in a female syconium would be able to oviposit in the long styled flowers. There would therefore be only a hundred eggs laid while several hundred seeds would be developing and therefore there would be no successful offspring production. This result could be partly predicted from data on monoecious figs. In a monoecious fig, emergence of the wasps from a syconium should always precede its ripening as the sweetening makes it sticky and seed dispersers consume the ripe fruits. Galil et al., (1973) have demonstrated that, in **Ficus religiosa**, the climacteric ripening of the syconium was triggered by the emergence of the wasps. This seems to occur in most monoecious species. Dioecious species cannot have a ripening system based on wasp emergences as the syconia that should become sweet do not produce wasps. It is therefore quite logical that, in dioecious figs, when a syconium produces a large number of seeds, the seeds determine when the syconium ripens irrespective of the stage of development of wasps if any are present. Even if a single female wasp manages to oviposit in a female syconium no wasps will be produced as long as pollination also occurs. A protection system against oviposition on female trees based on style lengths can therefore work in dioecious figs as there is no selection for longer ovipositors.

5. Why actively pollinate?

Many fig tree pollinators have an active behaviour of loading and unloading pollen. This behaviour is time consuming and can be lost, transforming the pollinator into a parasite, as we have seen for **C. galili.** It can therefore only be maintained through permanent selection.

In monoecious figs, Galil and Eisikowitch (1971) have shown that females that did not bear pollen produced mostly male offspring. They assumed that lack of pollination within a syconium induced food shortage for the larvae.

Such a food shortage would have led to a differential mortality of females and males. The evidence is however not completely satisfactory as fig tree pollinators have very complex sex ratio strategies and the lack of pollen may induce uncharacteristic results.

More recently, Verkerke (in prep.) has shown a possible function for pollination in a monoecious fig, **F. ottoniifolia**, confirming previous observations on **F. religiosa** (Johri & Konar, 1956). In this species, each flower into which an egg is laid is also pollinated and the embryo achieves its first divisions before being eaten by the wasp. So we may expect that this ensures a better larval nutrition than if no pollination had occurred. This may explain why a wasp preferentially pollinates the part of the syconium where it is laying eggs.

There is a very strong evolutionary argument confirming the role of early development of the parasitized fig embryo. In dioecious figs it is

generally admitted that male syconia cannot produce seeds (**Ficus carica** would be an exception, but it is often regarded as a cultivated tree and therefore data on it would not apply to other species; however it is one of the few species for which pollination is passive). According to Berg (1984), the female flowers in male trees are gall flowers and their pistils are not functional and should be called pistiloids. The basic argument is that he has never observed any seed in male syconia.

If we apply evolutionary arguments we get opposite conclusions. Many pollinators of dioecious figs are active. This is only possible if they benefit from pollinating. As they have no offspring in female trees, pollen has to have some effect in the male syconia where they reproduce. We may therefore predict that the female flowers of "male" trees are usually pollinated and even fertilized. The situation would be analogous with the one in monoecious figs. This hypothesis can be tested with microscopy. The theoretical argument is however very strong.

6. Conclusion

Manifestly most of the questions concerning the evolutionary stability of the symbiosis between figs and their pollinators have yet to be answered. The population genetics approach appears to be rather interesting. It enables the explanation of some of the observations and allows some new hypotheses to be tested. It also allows some previous explanations to be rejected.

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CALOTROPIS PROCERA **(AIT.) AIT. F. (ASCLEPIADACEAE) AND** XYLOCOPA **SPP.: A STUDY OF INTER-RELATIONSHIPS**

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1. Introduction

Calotropis procera (Sodom-apple) is a Sudanian and East Saharo-arabian evergreen shrub. In Israel, It grows especially in the Dead Sea oases and along the Arava Valley towards Eilat (Zuhary, 1966).

Preliminary reports from Israel (e.g. Gerling et al., 1983; Eisikowitch, 1986) have pointed to a high association between **Calotropis procera** and Carpenter bees. These, in conjunction with the known complexity of the flower stucture and the extreme weather conditions, led to the present investigation of the flower-bee interaction.

2. Materials and methods

Field observations were made during the summers of 1980-1985, at Hazeva, in the Arava Valley of Israel. Flowers were tagged, marked and observed hourly, between 5 am and 6 pm. Records were also kept of the temperature, relative humidity and pollinators' behaviour.

Nectar was withdrawn with microcapillaries and its sugar concentration immediately assessed with a pocket refractometer (product of Bellingham & Stanley, Tunbridge Wells, U.K.) from the concentration of equivalent sucrose solution. Bagging of flowers was done with bags made of organdy enables free movement of air.

Pollen germination was effected under laboratory conditions by the hanging drop method (Galil & Zeroni, 1969) but instead of using artificial solution, nectar was used as germinating media.

Within the glass tube a crop of sucrose was added, in concentrations that varied between 15% and 30%. The final hanging drop concentration was varied according to its volume, its original concentration and the vapour pressure created by the sucrose drop added.

3. Observations

The flowering period of **Calotropis procera** in Israel usually occurs between March and September. Nectar is usually available before flowers are open and ceases by the end of flowering. The flowers may open at any hour of the day and remain open for 2 - 3 days.

Principally, the flower of **Calotropis procera** is not different from other flowers of this family (Muller, 1883; Kerner, 1902; Galil & Zeroni, 1965; Macior, 1965; Judd, 1967; Myatt, 1976; Wantrop, 1974). The nectar is produced in the stigmatic chamber which connects to the nectar containers (cuculli) via spongy, diffused channels (Fig. 1, 2).

Unlike other Asclepiadaceae, nectar in **Calotropis** is concealed in the cucullus which is an almost fully enclosed chamber, Walled by relatively thick tissue, with a very narrow, hairy opening (Mantrop, 1974; Jaeger, 1971). The flower is very stout and no insect except the pollinators can approach its nectar.

4. Pollination and pollinators' behaviour

Flowers of **Calotropis procera** are visited in Israel exclusively by two Carpenter bees, **Xylocopa pubescens** and **X. sulcatipes.** The two Carpenter bees exemplify two types of behaviour: (a) "Nectar visits" and (b) "avoiding visits".

In "nectar visits" bees approach the flowers, land, insect their strong proboscis into the cuculli and, at the same time insert their legs into the stigmatic chamber and later, in the course of withdrawing their legs, the Carpenter bees frequently and fortuitously extract the paired pollinia from the flower. The extracted paired pollinia commence to rotate, change their orientation and adhere firmly to one of the numerous bristles on the bees'leg.

When the bees, with pollinia loaded legs, now repeat this behavioural pattern on new flowers, the paired pollinia, in certain cases, become inserted into the stigmatic chamber of the new flower, where they soak in the nectar secreted within (Fig. 2).

Usually when bees are preforming "nectar visits", the bees leave on the flowers corolla a scented substance secreted from their Dufour glands (its chemical character was studied by A. Hefetz). When a Carpenter bee (no matter which species) approach to those marked flowers, just before landing they turn away abruptly and this is thus manifested as the "avoiding behaviour" (Frankie & Winson, 1977).

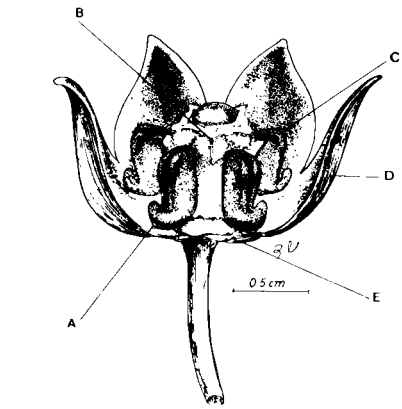
Normally when these bees are foraging they move from one flower to another and can make over 30 visits on the same plant (mean 12.45 $\pm$ 10.2). However, when they approach marked flowers they will make a few trials (mean 4.2 $\pm$ 2.4) and then turn to another plant (n = 50).

5. Experiments and results

Experiment 1.

Since **Calotropis procera** is exposed to very hot and dry conditions, it was of interest to ascertain to what exent the nectar retains its concentration and thereby its availability for the visiting bees. To this end, 20 umbels of flowers were bagged and tagged on the eve of the experiment; next day 10 bags were taken off and the flowers were observed, their nectar concentration measured once hourly on 5 flowers from each batch, visiting bees counted for 10 minutes every two hours, and temperature and relative humidity was recorded during the whole experiment.

Results show that protected flowers kept their nectar concentration almost constant during the day, while those exposed to visits displayed fuctuations in nectar concentration ranging between 30% - 60%. Bees' visits ranged from between 6.00 am to 6.00 pm, while **X. pubescens** visits, compared to those of **X. sulcatipes,** begin earlier during the lower temperature periods (Fig. 3).



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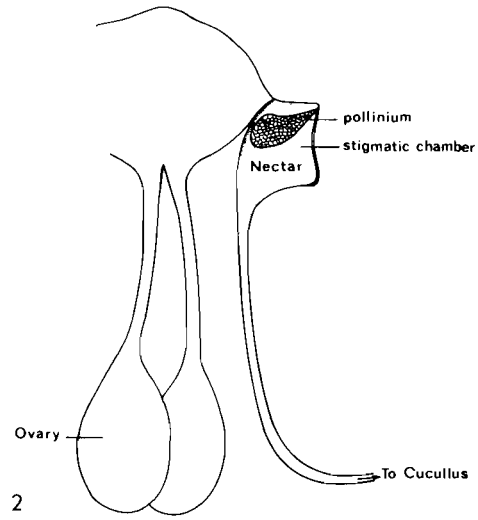

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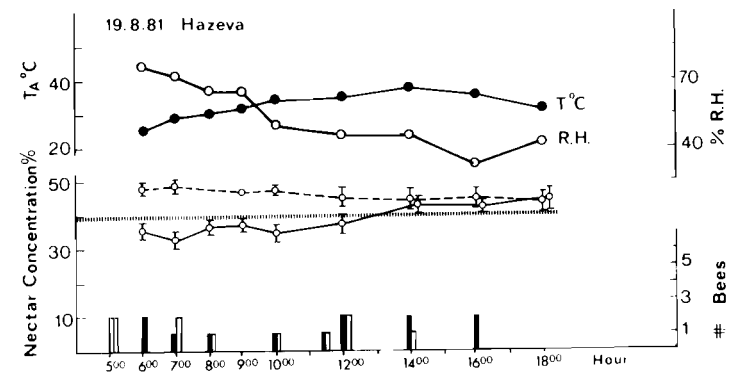

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Figure 1. Flower scheme of **Calotropis procera**. A: Cucullus; B: corpusculum; C: entrance of the Cucullus; D: petal; E: sepal.

Figure 2. Scheme of longitudinal section of the flower.

Figure 3. Bees' activity and nectar concentration on **Calotropis procera** in Hazeva.
T°C - Temperature; R.H. - Relative humidity; black bars - **X. sulcatipes;** open bars - **X. pubescens;** solid line - nectar concentration in open flowers; dotted line - nectar concentration in bagged flowers. Note constant horizontal line showing pollen germination threshold.

Experiment 2.

As demonstrated above, pollinia, as they are inserted into the stigmatic chamber, must become dipped in nectar whose concentration varies considerably. This experiment therefore was designed to ascertain to what extent pollen grains can successfully cope with various nectar concentration. Flowers of **Calotropis procera** were accordingly transported to the laboratory. An amount of about 15 ul nectar was removed from each flower and was laid on the cover glass. Two hours later, after equilibrium had been reached, the cover glass was raised, a pair of pollinia soaked in the nectar drop and the cover glass sealed again immediately. Five hours later germination of pollen and concentration of the nectar were assessed.

Results show (Fig. 3) a clear-cut phenomenon. Up to a concentration of nectar equivalent to 40% sucrose, pollen was almost 100% germination. Above this threshold the pollen remained without any germination.

6. Discussion

As already pointed out, **Calotropis procera** is linked (in Israel) to 2 Carpenter bees, which are totally dependent upon **Calotropis procera** as a source of nectar in the summer.

Figure 3 shows that those flowers which remain bagged and protected from visiting bees retained a higher nectar concentration. Such high nectar concentrations are above the pollen germination threshold. Flowers which were exposed to visiting bees had a lower and more variable nectar concentration (see also Raw, 1953). Such reductions bring the germinating media to a situation that enables pollen germination. Scent marking by Carpenter bees, which creates the "avoidance behaviour" has already been mentioned by Pijl (1954), Ramakrishna & Arekal (1979), Wantrop (1974), Frankie & Winson (1977), Winson et al. (1978), Hefetz (personal communication). Our results confirm the phenomenon that females are repelled from flowers recently visited not only by conspecific females, but also by another species. Such behaviour can explain how bees refrain from returning to already exploited flowers, and choose just flowers more rewarding both in amount and sugar value.

Again, such behaviour may also help the establishment of territories in the foraging area, since, as mentioned, bees tend to leave the area if they come across a certain number of marked flowers. From the plants' point of view, such markings, as suggested by Frankie & Winson (1977), increase inter-movements of foraging, which in turn raises outcrossing possibilities.

In conclusion, **Calotropis procera** is the only source of nectar and bee-bread for Carpenter bees in the extremely hot summer in Israel. The bees, in turn are the sole pollinator on the one hand and also indirectly improve germinating media on the other hand.

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RELATIONSHIPS BETWEEN MIMOSESTES (COLEOPTERA) AND ACACIA (LEGUMINOSAE): IS THERE COEVOLUTION BETWEEN THESE GENERA?

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1. Introduction

Larvae of the seed beetle family Bruchidae feed only in seeds, but most (85%) are in the family Leguminosae. Seeds of 32 other families are fed upon as well, especially the Palmae and Malvaceae. Females oviposit on seeds or pods, the small, first-stage larva burrows through the pod valve and/or seed coat, feeds, molts several times, and usually pupates inside one seed. The adult emerges from its pupal chamber and leaves a typical, round exit hole.

Most of the research that has been conducted on the species in the genus **Mimosestes** has been toxonomic although the more recent papers have included host records (Pfaffenberger & Johnson, 1976; Kingsolver & Johnson, 1978; Johnson, 1983). This is a non-economic genus that has a primary distribution from the United States to northern South America although some species are known from Brazil.

Species in **Mimosestes** are very specific to host genera and most species of **Mimosestes** are adapted to feeding in the seeds of species of **Acacia**. This can be explained in coevolutionary terms because most of the hosts of **Mimosestes** have thick pod valves that are more or less indehiscent and the species of **Mimosestes** have temporarily overcome this defense that excludes most other bruchids. The seeds of these plants are not exposed until the valves decompose, usually on the substrate beneath the parent plant or the pods are eaten by large vertebrates, the pod valves are digested and the seeds are defecated and thus exposed. Species of **Mimosestes** have evolved mechanisms such as eggs that are usually glued tightly to the thick pod valves and larvae that are adapted structurally and behaviorally to penetrate the valves. These enable them to enter the fruit and feed in the seeds. Conversely, those species of **Acacia** that have thin pod valves are usually fed upon not by **Mimosestes** but rather by species in the genus **Merobruchus** which apparently use a different set of adaptations to enter and then feed upon the seeds of their hosts.

Most species of New World acacias that I have collected have thick pod valves, suggesting that thick valves protect them from most seed beetles. Other species of **Acacia** are partially dehiscent and retain the seeds which are covered by a thick pulp inside the opened pod. The seeds appear to be displayed in the open valves to attract birds of other vertebrates to eat the pulp and thus disperse the seeds. The pulp may also be a defense against bruchids that oviposit only on exposed seeds. **Acacia gentlei, A. hindsii,** and **A. collinsii** fruits all have this kind of behavior (Table 1).

2. Methods

About 650 seed samples of various species of **Acacia** from the United States, Mexico, and Central America and the bruchids that emerged from their seeds were examined to arrive at the results included in this paper.

After examining the seed pods of the plants (Table 1), I classified the pods as having thick, indehiscent pod valves and remained indehiscent, at least while they were still attached to the plant (I); other pods had thin valves and were dehiscent to partially dehiscent (D); and a few pods had more or less thick pod valves which were partially dehiscent and the seeds were covered by an edible pulp which tended to hold the seeds together (C).

I then classified the bruchids as being generalists or specialists based on the number and kind of hosts that they attacked. If they fed upon five or more hosts than they were classified as generalists. Specialists fed upon no more then three hosts.

3. Results

Fourteen of the 16 species of bruchids fed mostly or exclusively in indehiscent pods. Others fed in seeds in category C (Table 1).

Thirteen of the 16 species fed in at least one species of **Acacia** while **M. enterolobii, M. protractus,** and **M. ulkei** do not feed in acacias. They were included in the study even though all are slightly aberrant examples of **Mimosestes.** Fully 74% of the hosts of **Mimosestes** are acacias.

Of the 16 species of burchids studied, only four are considered to be generalists. Two of the four (**amicus** and **mimosae**) feed in other genera in addition to **Acacia.** All of the genera of hosts are in the Mimosoideae except for **Cercidium, Parkinsonia,** and **Caesalpinia,** which are in the Caesalpinioideae.

Most adult females oviposit by gluing single eggs on the surface of seed pods (**acaciestes, amicus, humeralis, janzeni, nubigens, protractus, ulkei, viduatus**). Others may glue single eggs or eggs in clusters that overlap each other (**cinerifer, enterolobii, mimosae**) to the pod valve, presumably this latter behavior allows the eggs on the bottom protection from parasites and once one larval entry hole is made, the remaining larvae conserve energy by also using the first entry hole. Apparently there is no correlation between the thickness of the pod valve and the incidence of this latter behavior.

First-stage larvae of most **Mimosestes** (**amicus, cinerifer, enterolobii, insularis, mimosae, nubigens, protractus, viduatus**) enter the pod directly through the bottom of the egg which is glued to the surface of the pod. Larvae of some species (**acaciestes, humeralis, janzeni, ulkei**) emerge from the egg and crawl about in search of a suitable place to enter the pod or seed.

The larvae of all species usually feed in only one seed during the course of their development and then pupate inside that seed. In most instances the adult exits through the pod valve.

4. Discussion

Johnson (1981a) reported that there are three guilds of bruchids. One

guild glues its eggs to immature or mature pods (e.g. **Mimosestes, Merobruchus**); another oviposits upon mature, exposed seeds in partially dehiscent pods on the plant; while yet a third guild oviposits only on seeds after they have fallen to the substrate and are not covered by the seed pod. Bruchids in this latter category often oviposit on seeds exposed in cattle dung.

Seeds of species of New World **Acacia** are fed upon by more than 30 species of bruchids at some stage of their development and dispersal. Most are species of **Mimosestes** (13) and **Stator** (13).

According to Janzen and Martin (1982) the fleshy pulp of fruits were evolved to attract dispersal agents for seeds. If this is the case then the woody valves of legume fruits were probably evolved to attract large vertebrates to feed on the fruits, digest the fleshy or woody portions and then disperse the seeds in their feces. They also suggest that insects with the behavior of species of **Mimosestes** should enter, feed, and leave the seeds as soon as possible to avoid being eaten by dispersal agents. They hypothesize that the fruits with thick coverings are primarily coevolved with dispersal agents. I believe that bruchid beetles may have an impact also as most bruchids which have had these fruits available to them in time and space do not feed in them.

Because most of the plants that are hosts for **Mimosestes** have their seeds fed upon by species of **Stator** once the seeds are free of the seed valves, perhaps the bruchids are in fact in a coevolutionary race with the hosts and the dispersal agents. Species of **Mimosestes** (and other bruchids that oviposit on the pod valves) must feed rapidly and exit the seeds before the dispersal agents ingest the seeds. Once the exposed seeds have been voided by the dispersal agent those bruchids that oviposit on exposed seeds on the ground may attack the seeds. If the seeds are carried far from the parent plant the bruchids would have a very difficult time locating the exposed seeds unless chemical cues attract them to the dung of the dispersal agent.

Since the seeds of a species of acacia are rarely attacked by more than three species of bruchids, it appears that the array of defensive devices that a species of plant possesses prevents the great majority of bruchids from feeding in their seeds. The thick pod valves apparently prevent species of **Merobruchus** from entering these fruits and the chemicals in the seed coats and inside the seeds probably prevent many more bruchids from feeding in seeds of **Acacia**. Southgate (1978, 1979) pointed out that acacias are abundant in Australia but that there are apparently very few bruchids that feed in their seeds. He documented research that indicated that seeds of some Australian acacias have different chemical components in them than those from other parts of the world, thus suggesting that toxic chemicals account for the lack of bruchids in these seeds.

Johnson and Slobodchikoff (1979) and Johnson (1981b) found that 82.5% and 70% of bruchids in the genera **Sennius** and **Acanthoscelides**, respectively, are specialists. **Mimosestes** is similar because 75% are specialists.

Thus bruchids and acacias appear to be in a series of coevolutionary interactions with the stucture and chemistry of fruits and seeds of **Acacia**

because most bruchids do not feed in their seeds, some bruchids feed in exposed seeds (**Stator**) and others (**Merobruchus**) feed only in those with thin valves.

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Table 1. Species of **Mimosestes** and their hosts. I = plants with thick valves, more or less indehiscent; D = plants with thin valves, dehiscent or partially dehiscent. C = plants with valves that are partially dehiscent and the seeds are covered by an edible pulp that also holds them together and to the valve.

	Mimosestes *spp.*	*Host Plants*	*Valve Behavior*
1.	acaciestes	Acacia amentacea	*I*
		A. berlandieri	*I*
		A. constricta	*D*
		A. rigidula	*I*
		A. vernicosa	*D*

2.	amicus	Acacia constricta	*D*
		A. cymbispina	*I*
		A. farnesiana	*I*
		A. pennatula	*I*
		Cercidium floridum	*I*
		C. microphyllum	*I*
		C. praecox	*I*
		Parkinsonia aculeata	*I*
		Prosopis juliflora	*I*
		P. palmeri	*I*
		P. velutina	*I*
3.	anomalus	Acacia chiapensis	*I*
		A. globulifera	*D*
		A. pennatula	*I*
4.	brevicornis	Acacia gentlei	*C*
5.	cinerifer	Acacia cornigera	*I*
6.	enterolobii	Enterolobium schomburgkii	*I*
7.	humeralis	Acacia cymbispina	*I*
		A. pennatula	*I*
8.	insularis	Acacia farnesiana	*I*
		A. tortuosa	*I*
		Prosopis juliflora	*I*
9.	janzeni	Acacia cochliacantha	*I*
		A. cymbispina	*I*
10.	mimosae	Acacia cochliacantha	*I*
		A. cymbispina	*I*
		A. farnesiana	*I*
		A. globulifera	*D*
		A. hindsii	*C*
		A. pennatula	*I*
		Caesalpinia coriaria	*I*
		C. sclerocarpa	*I*
11.	nubigens	Acacia cochliacantha	*I*
		A. cornigera	*I*
		A. farnesiana	*I*
		A. globulifera	*D*
		A. schaffneri	*I*
		A. tortuosa	*I*
12.	obscuriceps	Acacia cornigera	*I*
		A. sphaerocephala	*I*
13.	playazul	Acacia collinsii	*C*

14.	protractus	Prosopis juliflora	*I*
		P. laevigata	*I*
15.	ulkei	Cercidium floridum	*I*
		Parkinsonia aculeata	*I*
16.	viduatus	Acacia chiapensis	*I*
		A. collinsii	*C*
		A. cornigera	*I*
		A. cymbispina	*I*
		A. gentlei	*C*
		A. globulifera	*D*
		A. hindsii	*C*

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THE ROLES OF PLANT CHEMISTRY IN ASSOCIATIONS BETWEEN SWALLOWTAIL BUTTERFLIES AND THEIR HOST PLANTS

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1. Introduction

The host plants of related species of oligophagous insects commonly share secondary compounds of the same chemical classes even though the host plants themselves may not be close taxonomic relatives of one another (Dethier, 1941; Ehrlich & Raven, 1964). Such patterns cannot be explained simply by parallel cladogenesis. Rather, they indicate that adaptation by an insect to particular secondary compounds in one host plant species confers preadaptation to colonizing other plants containing similar compounds. The nature of such adaptation and preadaptation, however, has remained unclear.

Following Fraenkel (1959), Ehrlich and Raven (1964) suggested that many secondary compounds evolved as plant defenses and that these have been overcome variously by insects in evolutionary time. Loss of sensitivity to the deterrent effects of a compound, and/or tolerance of any toxic properties it formerly possessed, would facilitate colonization of any plants containing the compound, regardless of botanical affinity. By contrast, Dethier (1941) and Jermy (1976, 1984) have proposed that chemical similarities among the host plants of related insects result from preadaptation in response to attractants or behavioral stimulants: colonization of novel host plants by an insect population will be more likely if such hosts contain compounds that the insects already use as cues for host recognition.

Here I review briefly some research undertaken to evaluate the above hypotheses as they apply to the Papilionidae (swallowtail butterflies), a family that contributed to the ideas of both Dethier (1941) and Ehrlich and Raven (1964). The family consists of about 560 species that, between them, attack plants in more than 25 families (Scriber, 1984). Despite their botanical diversity, subgroups of swallowtail food plants are linked with one another by their common content of various classes of secondary compounds, including coumarins, benzylisoquinoline alkaloids, and essential oils (Ehrlich & Raven, 1964; Feeny et al., 1983).

2. Plant chemistry and swallowtail behavior

Dethier (1941) showed that larvae of the black swallowtail, **Papilio polyxenes**, are attracted by several essential oil compounds from their umbelliferous food plants. Since the larvae will also feed on certain plants in the Rutaceae, the major host family of many **Papilio** species, and since several of the attractant compounds also occur in this family,

Dethier suggested that "the transition from one plant family to the other took place because of the presence of identical attractant chemicals in both families" (Dethier, 1941 p. 72). Later demonstration by Saxena and Prabha (1975) that larvae of **Papilio demoleus** are attracted to essential oil compounds present in their rutaceous hosts has provided further support for Dethier's hypothesis.

More recently, attention has shifted to the role of chemistry in oviposition behavior by swallowtails since oviposition "mistakes" by females provide the most likely route to colonization of novel host plants. There are numerous reports of female swallowtails laying eggs on host plants of other swallowtail species (Feeny et al., 1983).

Females of several swallowtail species can be stimulated to lay eggs on filter paper treated with alcoholic extracts of typical host plants. The tarsal contact stimulants contained in such extracts are generally polar and remain in the aqueous phase after extraction with organic solvents (Feeny et al., 1983). Ohsugi et al. (1985) separated the aqueous phase from extracts of **Citrus unshiu** into three fractions that stimulated oviposition by females of **Papilio xuthus**. The most polar fraction was active by itself but the other two fractions were active only when mixed together. The principal stimulants in these two fractions were identified, respectively, as vicenin-2 (a flavone glycoside) and N-methylserotonin (a tryptamine base). Other flavonoids, as well as the nucleoside adenosine, were found to have some stimulatory activity (Ohsugi et al., 1985; R. Nishida, personal communication).

Parallel work in our laboratory has revealed that the aqueous phase from extracts of carrot, **Daucus carota**, can be separated into three fractions that stimulate oviposition by **P. polyxenes** females. As found for **P. xuthus**, the most polar fraction is active alone while the other two fractions require combination to be stimulatory. The active component in one of these has been identified provisionally as luteolin-7-diglucuronylglucoside, a previously unknown flavone glycoside , while the other fraction contains at least two active bases (P. Feeny, K. Sachdev & L. Rosenberry, unpublished results).

That combinations of organic bases and flavone or flavanone glycosides have now been shown, surprisingly, to serve as contact stimulants for two **Papilio** species sugggests that these may be key classes of compounds to which all species of **Papilio** respond. Patterns of distribution of relevant classes of organic bases in plants are poorly known, however, and flavone glycosides occur widely (Harborne et al., 1975). Perhaps stimulants occur in non-host plants but are offset by the presence of repellents or oviposition inhibitors. Alternatively, flavonoids and bases may form part of a stimulatory chemical profile that also comprises compounds more characteristic of plant families exploited by swallowtails. In support of this possibility, we have found recently that oviposition by female **P. polyxenes** butterflies on artificial plants in free-flight cages is enhanced by addition of carrot volatiles, distilled from methylene chloride leaf washes, to the aqueous fraction containing the contact stimulants. Furthermore, electroantennogram preparations of **P. polyxenes** females are stimulated by several of the volatile components as they elute from a gas

chromatograph (E. Städler, P. Feeny & M. Carter, unpublished results). Though not yet complete, these experiments suggest that volatile essential oils may play a role in host-selection behavior by adults as well as larvae.

3. Plant compounds as chemical barriers to swallowtails

Though oviposition "mistakes" by swallowtails have been reported frequently, and must therefore be common in nature, shifts in host range are most unusual. This suggests that behavioral experimentation by adults or larvae, though necessary for initiation of host shifts, may not be sufficient to effect permanent colonization of novel host plants. One explanation for such a "bottleneck" in rates of new colonization lies in the realm of population genetics: genotypes of colonizing individuals may be disrupted by panmixis within the insect population at large, thus preventing a novel and heritable behavioral trait from becoming established through subsequent generations. This is especially likely when an insect's mating system lacks close linkage to the host plant (Gilbert, 1979), as is the case for **P. polyxenes** (Lederhouse, 1982).

A second, and not incompatible, hypothesis is that plants derive protection from would-be colonizers by virtue of various physical and chemical barriers to growth (Fraenkel, 1959; Ehrlich & Raven, 1964). Though repeated exposure to such barriers may eventually select for tolerant genotypes, as in the case of synthetic insecticides, lack of concomitant behavioral variation and restrictions imposed by population genetics could render such barriers effective over long periods.

In early experiments on this subject, we found that larvae of **P. polyxenes** could not survive on carrot or celery foliage that had been cultured with doses of allylglucosinolate found typically in cruciferous plants and that the effects could be attributed to toxicity rather than merely to feeding inhibition (Erickson & Feeny, 1974; Blau et al., 1978). This result provided support for the hypothesis that non-hosts contain chemical barriers to swallowtails.

As for possible barriers in swallowtail host plants themselves, Berenbaum (1978, 1981) showed that xanthotoxin, one of several linear furanocoumarins from the Umbelliferae, is highly toxic to larvae of the southern armyworm, **Spodoptera eridania**, but has no effect on growth or fecundity of **P. polyxenes**. Angelicin, one of the rarer angular furanocoumarins, affected the growth of **P. polyxenes** larvae only slightly but reduced dramatically the fecundity of female adults (Berenbaum & Feeny, 1981). Several benzylisoquinoline alkaloids from swallowtail food plants proved to be repellent and/or toxic to larvae of **S. eridania, Lymantria dispar** and **Hyphantria cunea**, all generalist lepidopteran species that do not normally feed on such plants. Aristolochic acid was particularly toxic to all three species (Miller & Feeny, 1983). Swallowtail larvae of several species were found to tolerate the alkaloids from their own host plants with no ill effects but showed varying degrees of sensitivity to alkaloids more typical of plants attacked by other swallowtails (Miller, 1986).

Though we have tested just a few of the numerous natural products present in swallowtail food plants, our results confirm that these plants contain chemical barriers to insect attack, consistent with the hypotheses of Fraenkel (1959) and Ehrlich and Raven (1964). That particular swallowtails can tolerate not only the compounds in their own host plants but also some that are novel to them shows that the barriers can be overcome in evolutionary time and that tolerance of one may confer preadaptation to tolerating others.

4. Plant chemistry and swallowtail survivorship

In addition to repellents and toxins, a variety of ecological barriers may face insects colonizing a novel host (Gilbert, 1979). For swallowtails, predation seems to be especially severe and was the major source of larval mortality in populations of **P. xuthus** feeding on rutaceous hosts in Japan (Watanabe, 1981) and of **P. polyxenes** on umbellifers in New York (Feeny et al., 1985). Even a slight increase in losses to predation following a shift to a novel host plant could prevent permanent colonization of that plant. Swallowtail larvae possess eversible osmeterial glands that release volatile deterrents (Eisner & Meinwald, 1965) and reduce predation (Damman, 1986). Larvae of several **Aristolochia**-feeding species sequester aristolochic acids from their food plants (Rothschild, 1972; Urzua & Priestap, 1985). When such defenses are dependent on particular classes of plant-derived compounds, as is the case for sequestration of aristolochic acid though apparently not for osmeterial secretions (Honda, 1983), selection may favor colonization of chemically-similar plants while inhibiting colonization of others.

Biogeographic evidence suggests that association between troidine swallowtails and the Aristolochiaceae is at least 50 million years old (Miller, 1986) - a striking contrast to the lability of many other insect-plant associations (Strong et al., 1984). The great age of this association may result from the unusual toxicity of aristolochic acids: the compounds have favored long-term survival of the plants and, at the same time, the insects have become trapped by dependence on the compounds for defense. Such specialization would presumably permit host shifts by troidine swallowtails within the Aristolochiaceae but has probably inhibited moves to plants of other families (Ehrlich & Raven, 1964; Miller, 1986).

5. Conclusions

Host selection, rather than being a rigid, deterministic phenomenon, is in practice a matter of probabilites. Though the plants attacked by an oligophagous insect will generally be those belonging to its current host range, there will always be a certain probability of attack on many other plant species in the insect's local environment. Colonization of a novel host can be considered as a sequence of stages, corresponding to barriers that an insect must overcome or bottlenecks that it must pass through. The combined probabilities of overcoming all the barriers may generally be very low but preadaptation at one or more stages can increase dramatically the chances of overall success. In the case of swallowtails, preadaptation

based on plant chemistry seems to have occurred at several stages in the colonization sequence, including initial acceptance of novel hosts (behavior), larval establishment (metabolism of toxins) and insect survivorship (chemical defense). Though the relative importance of the different kinds of chemical preadaptation must have varied from one host shift to another, it seems unlikely that overall patterns of chemical similarity among swallowtail food plants can be explained solely in terms of either behavior or adaptation to plant defenses.

Are the host associations of swallowtails forever constrained by plant chemisty? It seems that one extant species, the tiger swallowtail (**P. glaucus**), has escaped from chemical constraints to become the only generalized feeder among the swallowtails, attacking plants of more than a dozen families that display little taxonomic or chemical similarity. An important prerequisite for this escape seems to have been the ability of **P. glaucus** larvae, unlike those of Lauraceae-feeding relatives, to feed and grow successfully on the mature foliage of trees in temperate forests (Hagen, 1986) - foliage that generally lacks significant concentrations of toxins (Feeny, 1976). Such behavior, combined with relaxation of chemical constraints on female oviposition behavior and, perhaps, lack of dependence on plant chemistry for defense, may account for this unique example of generalized feeding in an otherwise oligophagous lineage (Hagen, 1986).

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SUMMARIES OF POSTER PRESENTATIONS

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THE PHYSIOLOGY OF COMPENSATION BY LOCUSTS FOR CHANGES IN DIETARY PROTEIN

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Previous work has demonstrated that fifth instar nymphs of **Locusta migratoria** L. respond to differences in levels of dietary protein by altering intermeal interval but not meal size (Simpson & Abisgold, 1985): insects fed a diet with 28% protein (P) eat the same sized meals less frequently than those fed a diet with 14% protein (p). The physiological basis for this compensatory response was investigated.

Insects fed the P diet had a significantly larger increase in blood osmolality during and after a meal than did those fed the p diet. Unexpectedly, this difference in blood osmolality did not result in a variation in the rate of gut emptying. Therefore a change in the rate of decline in negative feedback from gut stretch receptors does not underlie the alteration in interfeed interval.

40% of the difference in blood osmolality between p and P-fed insects was attributable to changes in blood free amino acid concentration. Of the 17 free amino acids found, 11 occurred in significantly higher concentrations in the blood of P-fed insects. There was no difference in the polypeptide/protein content of the blood of insects fed the p or P diets. Increasing either blood osmolality or free amino acid concentration by injection delayed the next meal, with the greatest effect occurring after injections which increased both.

These results suggest a mechanism whereby both blood osmolality and the concentration of various free amino acids regulate the time between meals, and thus compensatory feeding to changes in dietary protein.

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ROLE OF ENDOPHYTIC FUNGI IN ENHANCING HOST PLANT RESISTANCE TO HERBIVORES

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Plants of Gramineae, i.e., **Lolium perenne, Festuca arundinacea, F. longifolia** and F. **rubra** subsp. **commutata** harboring endophylic fungi (**Acremonium**, spp.) are deterrent to 18 insect species of a wide taxonomic range that includes Coleoptera, Lepidoptera, Hemiptera and Orthoptera (Ahmad et al., 1986; and references therein). In chewing insects such as **Acheta domesticus** and larvae of **Listronotus bonariensis** and **Spodoptera eridania,** acute toxicity is associated with the consumption of plants' leaf sheaths where the fungal level is highest. In addition, there is evidence for a highly polar antifeedant compound in **L. perenne** which supresses feeding of **L. bonariensis** adults and possibly also in a closely-related weevil, **Sphenophorus parvulus.** The antibiosis of sap-sucking Hemiptera that primarily feed on leaf blades suggests that some toxic or antifeedant allelochemical is translocated from endophyte infected area to other parts of the plant.

The identity and mode of action of allelochemicals that are causal for insect antibiosis and occasionally adversely affect vertebrate grazers is not fully known. The nature/manifestation of antibiosis appears to be herbivore and host specific. The suspected substances, either of fungal or plant origin, are: in **F. arundinacea**, ergot alkaloids and lolinetype pyrrolizidine alkaloids; and in **L. perenne**, a neurotoxic indole called Lolitrem B, and an antifeedant of unresolved structure. More research is needed to better understand the chemical basis of insect antibiosis and vertebrate toxicosis. Nevertheless, it is apparent that the endophytes are important sources of insect resistance which plant breeders can incorporate into new crop varieties.

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MORPHOGENETIC RESPONSES OF SOME SOLANACEAE INFECTED WITH THE GALL-MITE ERIOPHYES LYCOPERSICI W.

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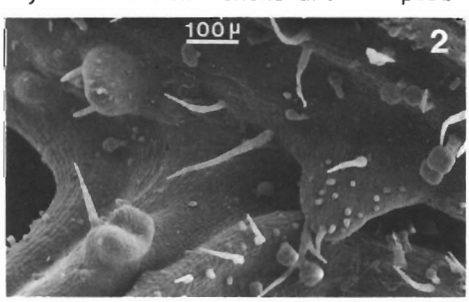
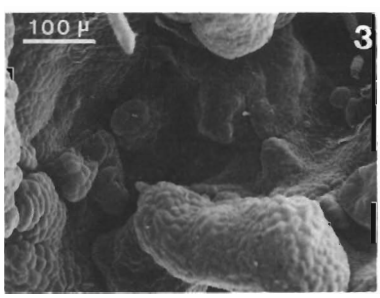
The mite **Eriophyes lycopersici** (Fig. 1) may provoke gall formation on a wide range of host-plants belonging to the Solanaceae family. The purpose of this study is to compare the morphological changes that occcur in the vegetative region of two plant-species, **Solanum lycopersicum** L. var. **cerasiforme** and **Nicandra physaloides** Gaert. after experimental mite infection.

In both species the main symptoms induced by the mite are the following: 1) inhibition of internodal expansion, 2) inhibition and deformation of leaf growth, 3) occasional stem fasciation, 4) development of supernumerary branches, 5) formation of a great number and variety of appendages on the leaves.

This study focuses on the development of these appendages (Fig. 2, 3). Many of them develop like warts or outgrowths, others like little leaves and still others like shoots. Intermediates between these types of appendages occur and morphological continua are distinguished according to different criteria. The appendages may be simple or branched, non vascularized or more or less vascularized, irregularly grouped or arranged in a more or less phyllotactic pattern, separate from each other or more or less continuous with each other. Much of the observed variation is continuous.

The two plant species respond, however, differently to mite infection. In **S. lycopersicum** (Fig. 2) the development of warts and of shoots on leaves occur more frequently. In **N. physaloides** (Fig. 3) the development of more or less vascularized outgrowths is more frequent, and in this species the gall symptoms n°1 and n°4 are more severe. Moreover, in **N. physaloides** symptoms persist during all the life-time of the host plant, while in **S. lycopersicum** natural recovering and reversion to normal plant morphology may occur a few months after experimental infection.





A CASE OF STRICT CHEMICAL DEPENDANCE: ALLIUM - THE LEEK-MOTH - ITS ENTOMOPHAGE

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Mining larvae of the leek-moth, **Acrolepiopsis assectella**, develop only on **Allium** plants. Young pupae of this lepidoptera are parasitized by the wasp **Diadromus pulchellus** (Ichneumonidae). The effects of **Allium** chemicals were studied on the host plant search by the moth, on its oviposition specificity by contact stimulus and on the feeding of its larvae. Next, they were studied on the search of the host-pupae and its habitat in the parasitoid wasp.

Allium produce allelochemicals, someones of which are well-known, particularly those coming from the four very specific S-alkylcysteinesulfoxides, the relative abundance of which fluctuates according to the species.

The leek-moth is attracted by them, mostly by the labile propanethiosulfinic acid-S propyl ester specially emitted by the leek. The moth is also attracted by some of the C6 general green leaf volatiles which are very common. The non volatile stable specific chemicals which stimulate the moth egg-laying and vitellogenesis are present in very little quantity on the leaf surface and are perceived at the tarsus level by the females. They do not belong to the metabolic pathway of the sulfur compounds and nevertheless their specificity is very important too, because the moth oviposits only on the **Allium** genus and specially on the leek.

The leek-moth larval feeding is also stimulated besides sugars by the specific sulfur chemicals, namely non volatile precursor amino-acids.

The locomotor activity or kinesis of the wasp are increased by leek odour, mostly by the emanation of the leeks damaged by the moth larvae. The stable sulfur compounds which appear when sulfinothioic acid-S-esters disproportionate, i.e. disulfides and sulfonothioic acid-S-esters, are responsible of the stimulation.

Otherwise, sulfur volatiles specifically produced by **Cruciferous** species stimulate these wasps too and this fact re-opens the question of the wasp specificity for the leek-moth. In some accurate conditions for the wind, this parasitoïd is also attracted by damaged leeks and this can be observed not only for the labile sulfur volatiles but also for the more stable compounds they yield later on.

We can notice on each level of the trophic chain an action of the allelochemicals emitted by the primary producer, the **Allium** but these actions are more or less specific of a particular structure depending on the studied activity: the labile sulfur volatiles are informative molecules responsible for a great part of the orientation of the insect studied while the stable sulfur volatiles play more diverse roles and while some behaviours like the moth oviposition need the presence of very specific substances, that differ from the previously identified ones.

Though, so interactive and so characteristic cases are very rarely studied, this type of chemical communication occurs in numerous other trophic chains.

APHID BIOTYPES IN RELATION TO HOST PLANTS

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A biotype is usually defined as an individual or population that is distinguished from the rest of its species by criteria other than morphology, for example, a difference in parasite ability. Aphid biotypes in relation to host plant resistance have been reported in at least 12 species, and they may differ in their feeding behaviour, digestive enzyme activity, growth, reproduction, survival, nutritional requirements, polymorphism, virus transmission, insecticide resistance, isozyme patterns, and other characteristics. Examples of variability are given using the pea aphid, **Acyrthosiphon pisum** (Harris), where seven allopatric clones were found to differ among themselves in many of the above characteristics. For instance, growth and reproduction on pea plant, **Pisum sativum** L., susceptible variety Lincoln, differed significantly among clones J, T, K, N and C; J, T, and K had faster growth and higher reproduction than N or C, mean reproduction per female of C being 4.7 larvae/day compared to 9.6 for J (Auclair, 1978). Mortality of C after 10 days on either pea plant or a chemical diet was significantly higher than that of K or J, whereas clone N gave intermediate values, and growth and feeding rates on the diet was lowest for C and highest for J. Five amino acids were essential for growth and/or reproduction of J whereas seven were essential for C (Srivastava et al., 1985). Isozyme profiles determined by vertical polyacrylamide gel electrophoresis differed between J and C concerning esterases and superoxide dismutase (Simon et al., 1982). Propensity for alatae production was highest in Lg, moderate in C and Lp and low in J (Auclair & Aroga, 1984), whereas tolerance to lower temperatures was highest for J and Lg, moderate for Lp and low for C. These results demonstrate once more the great intraspecific variability in **A. pisum**.

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BIOLOGICAL ASPECTS OF THE PLANT-ANT RELATIONSHIPS IN THE RAIN FOREST: ANT-GARDENS IN FRENCH GUIANA

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In Guiana, on the trunk and at the top of trees, in light localization, some epiphytic plants live in association with ants. They compose ant-gardens (Wheeler, 1921; Kleinfeldt, 1978; Madison, 1979). The principal plant species are: **Anthurium gracile** (Rudge) Lindl., **Philodendron melinonii** Brong. (Araceae), **Aechmea mertensii** Schult., **Streptocalyx angustifolius** Mez. (Bromeliaceae), **Codonanthe calcarata** (Miq.) Hanst., **C. crassifolia** (Focke) Morton (Gesneriaceae), **Peperomia glabellae** Griseb. (Piperaceae). Other species are occasional plants: **Araeococcus micranthus** Brongn. (Bromeliaceae), **Epiphyllum phyllanthus** (L.) Haw (Cactaceae), **Ficus myrmecophyla** Warb. (Moraceae), **Epipendrum** sp. (Orchidaceae), **Polypodium ciliatum** W. (Polypodiaceae), **Marckea coccinea** Rich., **M. formicarum** Damm. (Solanaceae). Some ant species are **Camponotus** but others are presently being described. The ant nests arise from a dense root system of one plant species. Three types are detected: Araceous type in which nest structure includes the root system of **Philodendron melinonii** with tenuous roots or **Anthurium gracile** (adventious root system with velum as Orchidaceae), Bromeliaceous type where "woof" is constitued by **Aechmea**'s or **Streptocalyx**'s root system, at last, Gesneriaceous type where **Codonanthe** is alone in the beginning of nest. The different species which compose ant-garden structure are divided in emergent-plants (Araceae, Bromeliaceae, Moraceae, Solanaceae...), overlapping plants (Gesneriaceae, Polypodiaceae) and hanging plants (Piperaceae, Cactaceae).

Each ant-garden functions as an ecosystem. Ants are finding their food in pulpy fruits (except **Peperomia** all epiphytic plants acting as ant-gardens have pulpy fruit) but also in sugar secretions (foliar nectaries of **Codonanthe**, secretory elements of **Philodendron melinonii**). The part of ants, albeit occasional, is seed dissemination but birds have the first part in the beginning of ant-gardens.

The **Philodendron**'s roots have fungal mycelium into external cells of suberous zone. This mycorhizes have a part in organic substance absorption. Ant-garden humus is very rich in organic substance (80-90%).

An analysis of ant-garden populations in a pomelo plantation shows the dominant characteristic of Bromeliaceous type with **Aechmea** alone; sometimes, araceous type with **Anthurium** is present.

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THE RELATIONSHIP BETWEEN THE HALL SCALE, NILOTASPIS HALLI, (DIASPIDIDAE) AND ITS HOST PLANT: THE EFFECT OF THE PLANT ON THE SCALE

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1. Introduction

The scale occurs in Asia, North-Africa and the USA on the trunks, branches, buds and fruits of various **Prunus** and **Amygdalus** sp. (Borchsenius, 1966; Fosen et al., 1953). In Israel, three generations were found annually (Berlinger et al., 1984).

2. Methods

Sample unit. Samples of ten 1-year-old branches, 8-15 mm in diam, were taken and their ten basic buds examined for scales. Traps. A strip of black cloth, 5 mm wide, was wrapped around a branch. The scales that had settled under the trap, were counted.

3. Results

Spring activity of the scales was not affected by bud-break: 3.5% (January) and 95.8% (March) of all females with developed eggs, were found on sprouting trees, compared with 2.3% and 90.5% on dormant or just blooming trees, respectively. Eggs developed in 80% of the females within 19 days at 28°C, but none in control scales kept outdoors in January. More scales were found in closely attached buds (4.9 scales/bud) than in protruding buds (1.5 scales/bud). The same trend was found on the three cultivars examined (Table 1). The importance of buds as shelters for the scales is demonstrated by traps (Berlinger & Gol'berg, 1978) (Table 2).

Table 1. Relation between scale density and peach bud morphology (± S.D.)

	HERMOSA	SUWANNEE	SUMMERSET	Correlation
No. of scales/branch	72.6±11.9	53.2±12.4	11.9±4.7	
Scales on attached buds (% of all buds)	75.9± 6.3	69.3±12.5	22.2±5.3	r=0.89

Table 2. Distribution of the scales on a peach branch, in buds vs traps.

	Number of scales		
Peach cultivar and branch	in buds	in traps	Correlation
Hermosa, winter branch	24.4	74.5	
Hermosa, spring branch	15.1	60.0	r=0.8
Summerset, winter branch	14.9	35.1	

4. Concluding remarks

Activity of the scales in spring is renewed by temperature, and not by bud-break. Population density is affected by available shelter, viz., bud morphology or traps. The correlation between number of scales in traps vs.

buds renders the traps a useful tool for research and monitoring.

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ACTIVITY OF LIGNANS AS MIXED FUNCTION OXIDASES (MFO) BLOCKERS IN TWO HERBIVOROUS INSECTS: IN VITRO STUDY

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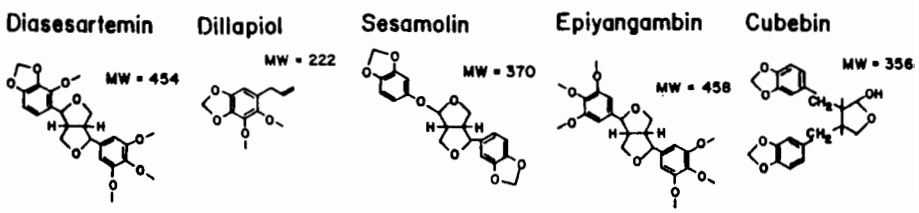
In plants, lignans (2 phenylpropanoid units linked by their central carbon), occur together with other toxic allelochemicals. The methylenedioxiphenyl group in some lignans (sesamoline), is known to decrease the level of the MFOs in the insect midgut wall, and therefore has been used as an insecticide synergist.

In the first step of the study, the effect of lignans on the enzyme activity was measured by the transformation rate of aldrin to dieldrin. The lignans assayed, diasesartemin, dillapiol, sesamoline, epiyangambin were extracted from the plant family of the Asteraceae, and cubebin was extracted from a Piperaceae.



Diasesartemin and dillapiol inhibited the activity of the MFOs from the European corn borer, **Ostrinia nubilalis**, by 50% and 70% respectively at the concentration of 10^{-5} and 10^{-4} M in the incubation medium. Sesamoline and epiyangambin were less potent inhibitors of these enzymes with 40% and 20% inhibition at 10^{-5} M. A slight inhibitory effect was observed in the preliminary assays with the MFOs of the tobacco hornworm, **Manduca sexta**. Cubebin caused no inhibition of the enzymes of either insect species.

The ability of some lignans from the Asteraceae to decrease the detoxification enzymes activity of their insect predators (**O. nubilalis** is a common host of the Asteraceae but not of the Piperaceae, whereas **M. sexta** does not include these families in its diet), suggests that lignans may play a role in plant self defense.

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A COMPARATIVE STUDY OF HOST PLANT ACCEPTANCE BEHAVIORS IN RHAGOLETIS FRUIT FLIES

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Our research on fruit flies within the genus **Rhagoletis** is focused on the evolutionary differentiation in host selection behaviors and survival abilities in newly formed races and species of phytophagous insects. Our field and laboratory studies have analyzed the host acceptance behaviors of two closely related sibling species; **R. pomonella** infests apples and the fruits of other plants in the Rosaceae and **R. mendax** is a specialist on blueberries (Ericaceae).

In our field behavioral studies we worked with young mated females which were released to either a McIntosh apples plant or Bluehaven blueberry plant inside a large plastic mesh cage. We developed a BASIC language program for a TRS-80, model 100 portable microcomputer to record the number of occurrences, starting times, sequence and duration of 14 different behaviors. The program also enables the observer to keep track of the position of the insect on the host plant (e.g., fruit, leaves or branches) under field conditions. A total of 166 female releases were made to blueberries and apples; 82 of these observations were on **pomonella** flies and 84 were on **mendax** flies.

On the fruits of blueberry and apple plants, **R. mendax** and **R. pomonella** show differences in the number of occurrences and duration of several key host acceptance behaviors. These behaviors include touching the surface of the fruit with the mouthparts, antennating, probing with the ovipositor, laying an egg in the fruit and dragging the surface of the fruit with the ovipositor while laying down an oviposition deterring pheromone. On Bluehaven blueberries, **mendax** females displayed these behaviors more often than the **pomonella** females. This difference between the species was reversed on McIntosh apples where the same set of behaviors were shown more frequently by the **pomonella** females. On blueberry fruits, **mendax** females laid over four times the mean number of eggs deposited by **pomonella** females. The opposite patterns was found on apples where the **pomonella** flies had a significantly higher rate of oviposition. In the sample of **mendax** females we have studies, there are none which oviposited in McIntosh apples in field trials.

Another aspect of the behavioral differences which exist between **mendas** and **pomonella** are differences in the frequencies of transition between behaviors and the presence or absence of entire sequences of behaviors. The conditional probabilities of transition from one behavior to another were computed from the TRS-80 data files. These were used to construct kinematic diagrams for the behaviors displayed on apple and blueberry plants. On apple fruits, **mendax** flies show distinctly different sequences of behavior compared to **pomonella.** The R. **mendax** females lack the entire behavioral sequence of touching the surface of the fruit with the mouthparts followed by antennating, probing, and dragging the ovipositor. The **pomonella** flies have higher probabilities of transition between these

host acceptance behaviors. The two species also show differences in the sequences of behaviors they display on blueberry fruits. The results obtained from these experiments and our prior viability studies show that the divergence of host selection behaviors and survival abilities are major components of the evolutionary differentiation between these sibling species of **Rhagoletis.**

AIR POLLUTION AT A MOTORWAY: EFFECTS TO APHID INFESTATION

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Several investigations at the verge of a motorway revealed increased aphid infestation on their host plants in different experimental designs (Dohmen, 1985; Braun & Flüeckiger, 1984). Contributive stress factors like drought or deicing salt did not explain the phenomenon entirely.

Figure 1. Fumigation chambers at the motorway.

However, fumigation experiments with filtered and ambient air (Fig. 1) on **Viburnum, Crataegus** and **Phaseolus** plants produced up to eight fold increases in the populations of **Aphis fabae** and **Aphis pomi** in ambient air (Braun & Flüeckiger, 1984; Bolsinger & Flüeckiger, 1984) and furthermore, shoot growth and leaf areas were found to be significantly reduced in accordance to other studies (Taylor & Eaton, 1966).

Air quality measurements made at the verge reported high half hourly mean values of NOx from 400 to 950 ppb. NOx is thought to act as a possible and additional nitrogen supply for plants and may change the nitrogen metabolism in plants (Ito et al., 1984). Thus, it is supposed that biochemical changes in particular of nitrogen compounds may lead to modifications of the plant-aphid relationship. Phloem analysis demonstrated considerable changes in total free amino acid content (Fig. 2) as well as in the pattern of amino acids. Certain amino acids like Gln, Asn and Arg were found to be significantly increased in the ambient air treatment. The altered pattern of amino acids will now be checked up on artificial diets to cover up the significance in aphid development.

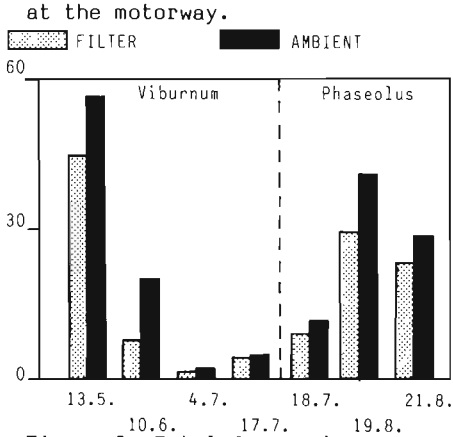

Figure 2. Total free amino acid content in phloem of fumigated plants.

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HAS THE ACANTHOSCELIDES OBTECTUS GROUP EVOLVED IN THE ORIGINAL ZONE OF ITS HOST PLANT (PHASEOLUS L.)?

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The bean weevil (**Acanthoscelides obtectus** (Say)) is a cosmopolitan polyvoltine insect; its larvae feeds in the dry seeds of the french bean (**Phaseolus vulgaris**). In Mexico, the original zone of its host-plant, a complex relationship has been found between **Acanthoscelides-Phaseolus** species. In the same geographical area, in the northern part of the state of Morelos, wild type populations and cultivars of **P. coccineus, P. vulgaris** (very close relatives) and P. **lunatus** are found. From the dry seeds of **P. coccineus** and **P. vulgaris, A. obtectus** and **A. obvelatus** emerge; from P. **lunatus** adults of **A. argillaceus. A. obtectus** also attacks cultivars of **Vigna unguiculata** and **Zabrotes subfasciatus** emerge from P. **vulgaris** and P. **lunatus.** The same bruchid species emerge either from wild type populations or cultivars of each host plant.

Inside the **Acanthoscelides obtectus** group differences between **A. obtectus** and **A. obvelatus** (very close relatives) can be found. In **A. obtectus:** the eleventh antennal segment is red orange, segments 7-10 broader than long, 8-15 petalloidal forms in the micropyle, different arrangement in the armature of the internal sac, cosmopolitan distribution, a weak and sometimes null quiescence in adults (part of the population can reproduce throughout the year if host plant is present, the rest will do it in the reproductive period). In **A. obvelatus:** the eleventh antennal segment is black to brown, antennal segments are narrower than broader, 3 petalloidal forms in the micropyle, distribution from Mexico to Colombia, obligatory diapause in adults. Different responses in the reproductive behaviour can tell us why **A. obtectus** became an important worldwide pest and not **A. obvelatus.**

The complexity of the relationship can be explained when in addition to the selective pressures (different responses in their reproductive behaviour) we add the third trophic level (parasitoid), which uses **Acanthoscelides** as host for their progeny. Six ectoparasitoids of larvae and pupae have emerged (**Horismenus** sp. ca. **depressus** Gahan, **Stenocorse bruchivora** (Crawford) **Eupelmus cushmani** (Crawford), **Torymus atheatus** Grisell, **Chryseida bennetti** (Burk) and Chalcidae No. 1, as well as one egg parasitoid (Trichogrammatidae, not yet identified). All these parasitoids are abundant in Morelos and don't appear to be specific to **Acanthoscelides** and its host plant. They are generalist bruchids and use them as alternate hosts. The parasitoids attack all instars of development, except for the adult. There is a sequence in the presence of the parasitoids.

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SCOTS PINE FOLIAGE AND DIPRION PINI L.

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1. Biology. Sawfly, two generations per year, larvae of the 1st feeds on old foliage, larvae of the 2nd on current year foliage.

2. Behavior and alimentary test. Juvenile foliage has antifeedant and nocious properties, decreasing until end of July, and a direct or indirect effects on diapause.

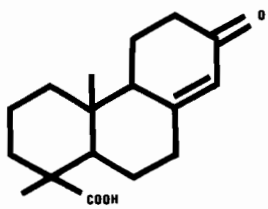

Fig.1: **NEOABIETONE**

3. Diterpenoid in resin acids. These facts are closed to the phenomenons observed by **Neodiprion** sawflies, in connection with the new foliage concentration in resin acids and particularly with the 13-keto 8-(14)-podocarpene-18-oic acid (Neoabietone) according to Canadian and Finnish researchers (Ikeda et al., 1977; Niemala et al., 1982).

Neoabietone was found with other resin acids in polar acid fraction extracted from **Pinus silvestris** L. foliage. This fraction decreases in the new foliage until the end of July, at that time it becomes acceptable. These compounds are extracted or synthetized and tested on **D. pini** populations, while their concentration is studied according to foliage development, pine species, and larval damages.

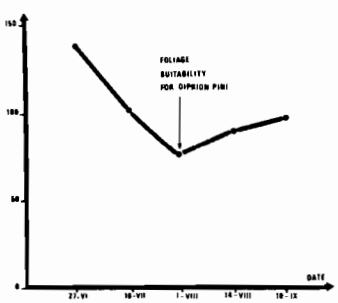

Fig 2 POLAR ACID FRACTION WEIGHT FOR 100 mg OF TOTAL LIPIDEOUS ACID FRACTION (mg)

4. Importance for Diprion **populations.** The outbreaks cause a strong foliage consumption in autumn, resulting in a scarce old foliage in the following spring. Then consumption of juvenile foliage as possible variations of resin acid concentrations in the foliage after defoliations induced mortality, physiological weakness, diapause, and the population regression.

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RESEARCH TO DEVELOP PLANTS RESISTANT TO THE COLORADO POTATO BEETLE, LEPTINOTARSA DECEMLINEATA (SAY)

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The genetic plasticity of the Colorado potato beetle (CPB) enabled it to develop resistance to nearly all insecticides in the northeastern United States. As a result, economic cultivation of potatoes, tomatoes, and eggplants in these areas is jeopardized. To overcome this threat we sought resistant **Solanum** spp. that were suitable for hybridizing with **S. tuberosum**. Accessions of **S. chacoense** were found whose alkaloids were primarily leptines, a type of glycoalkaloid toxic to the CPB that does not occur in tubers. One particular selection (PI 320287-1) contained 120 mg % fresh weight of leptines out of a total glycoalkaloid content of 159mg %. To ascertain if the CPB could adapt to this selection, neonate larvae from a laboratory colony were weekly placed on PI 320287-1 and those surviving to the 3rd larval instar were moved to tomato plants, our usual host plant for CPB rearing, to complete development. Then their offspring were placed on PI 320287-1 for 3 instars. This was continued for 12 months with the selected CPB maintained as a separate colony. With a test that uses the developmental stage of neonate CPB larvae as an indicator of plant toxicity, we found that 74% of larvae from the selected colony had developed past the 1st instar in 4 days on PI 320287-1 whereas only 28% of the laboratory colony larvae had gone beyond the 1st instar. On tomato plants at least 81% from both colonies had developed past the 1st instar. Subsequently we found that the selected colony could complete development to adults on PI 320287-1. Although the selected colony had the ability to survive in the presence of leptines, it had also acquired disadvantageous characteristics. When reared on tomatoes from egg to adult 42% completed development vs 73% of the laboratory colony; development time was 26.4 days with selected colony vs 23.9 days with laboratory colony. Egg production was higher by selected colony females, 1335 vs 962 per female by laboratory colony females but was over a longer period. That the CPB could rapidly adapt to leptine deterrency became evident when laboratory colony larvae were place on PI 320287-1 and the offspring of the survivors tested on PI 320287-1 and tomato. The 4th day after hatching 62% of the 1st generation larvae developed past the 1st instar whereas only 40% of laboratory colony were in the 2nd instar by day 4. The lack of vigor of the selected CPB strain also was indicated when the same populations were placed on tomato. By day 4 the laboratory colony had 100% past the 1st instar but the selected population had 91% past the 1st instar. It thus appears that the Colorado potato beetle had the genetic plasticity to adapt not only to man-made toxins but also to plant-made toxins. Development of plants that by themselves would provide adequate protection from the beetle seems unlikely; however, resistant plants could have an important place in an integrated management system.

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INFLUENCE OF FERMENTING PROCESS ON THE STRUCTURE OF THE BIOCENOSIS (DROSOPHILA AND PARASITIC WASPS) ASSOCIATED TO THE PRICKLY PEARS OF OPUNTIA

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The entomoparasitic biocenosis associated with decaying prickly pear in Tunisia involves the three primary consumers: larvae of **Drosophila melanogaster, D. simulans** and **D. buzzatii** and the two parasitic wasps: **Leptopilina boulardi** and **L. heterotoma** (Carton et al., 1986). We studied the gradual change of chemical composition during the rotting process and evaluated the levels of ethanol, pH (acetic acid?), fructose, dextrose and sucrose. The objective was to investigate the effects of the natural chemicals produced by the fermenting fruit and rotting cladode on the interaction between the parasitic wasps and their **Drosophila** hosts. The temporal sequence of egg laying by the three species of **Drosophila** depends on the stage of the fermenting process. The duration of egg laying by each species is longer on fruits (compared to cladodes): this is possibly due to the extended duration of fermentation of fruits. About the **Drosophila** development, the most striking results is that although each species lays eggs on cladodes only **D. buzzatii** is able to develop. Furthermore, larvae of **D. melanogaster** have a strong preference for medium containing ethanol. While larvae of **D. simulans** show no preference (Parsons & King, 1977). We demonstrated (Carton, 1978) that, like **D. melanogaster**, female parasitic wasps are also attracted to volatile fermentation products and that the degree of parasitism is highest in **Drosophila** larvae which feed in media with high levels of ethanol.

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PELARGONIUM **CULTIVAR SELECTION BY THE GREENHOUSE WHITEFLY**

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Differences in infestation by the greenhouse whitefly, **Trialeurodes vaporariorum** Westwood (Homoptera: Aleyrodidae) among three **Pelargonium x domesticum** cultivars, were related to several plant characteristics.

When whitefly adults could choose between the three cultivars they did not show any preference 12 hours after being released in the glasshouse. However, after 36 and 6 hours, more adults were found on cultivar 2.

Measurements of hairiness and epidermis thickness showed that the leaves of cultivar 2 had more glandular hairs and a thinner epidermis. Leaf colour, defined by the chromatic components LX and LY, was not clearly related to adult infestation.

The number of eggs laid by whiteflies, survivorship during larval development and the fecundity of emerging females on the three cultivars were not significantly different.

The results indicate that the observed infestation levels may be due to adult selection after landing on the plant.

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FORAGING BEHAVIOUR OF ANTS MESSOR STRUCTOR IN RELATION WITH THE CHARACTERISTICS OF THE SEEDS (HYMENOPTERA, FORMICIDAE)

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One large colony of **Messor structor** was studied in Touraine. Seed distributors (round boxes, 8 cm diam.) each with a photoelectric cell were placed on the foraging arena of the colony. Cells were connected to a graph-recorder which allows automatic recording of the passages of ants entering or leaving the distributors. These were filled with a constant number of seeds every week or day and the number of seeds retrieved could thus be counted.

Exp. 1: Influence of the seed species

It is known that **Messor** are fond of Graminaceae seeds but they can retrieve almost all seed species. Melon-seeds were offered in some distributors and rye-grass in others every week during 9 weeks. During the first period of 3 weeks 72% of the rye-grass and 48% of the melon seeds were collected and later almost all the seeds (99%). It confirms that ants do prefer some seed species, but after a period of habituation they retrieve all the available seeds, and the distributors are rapidly emptied.

Exp. 2: Influence of the seed size

3 sizes of rye-grass (3, 5 and 7 mm) were offered daily to the ants for 18 days. The harvest increased regularly from 26 to 97% for the largest seeds. These large seeds are slightly preferred to middle-size seeds but the difference is not significant. Small seeds were less transported (6 to 51%). When very large food sources are available the workers prefer the larger items, which could be interpreted as an optimization of the harvest.

Exp. 3: Influence of the quality of the seeds

Empty seeds were offered in some distributors, they were not transported where normal seeds were all collected. When the choice was between ripe and unripe seeds, there was only a slight preference for ripe seeds but it is not significant (88 vs. 79%). This can be explained by the fact that ants also eat unripe seeds when they are the only available ones in the beginning of the season.

Exp. 4: Selectivity on heterogeneous food sources

Full and empty seeds were proposed simultaneously in the same distributors. In this situation, 25% of the empty seeds were transported, which indicates a decrease of the selectivity (number of edible items retrieved/total number of items retrieved). The selectivity was correlated to the number of passages/day. This means that when the traffic increases ants retrieve more useless items, perhaps due to a phenomenon of facilitation.

Messor ants adopt a foraging group strategy which permits the complete and rapid exploitation of food sources, they are opportunistic. It is an adaptation to arid climates where a lot of seeds are available only during limited periods of the year. This could explain why harvesting ants could be a danger for homogeneous plantations of Graminaceae. For example, in the Mediterranean region when a lawn is sowed, it is necessary to destroy the **Messor** with a pesticide at the same time, otherwise all grass seeds are retrieved in the nests.

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TEMPORAL COINCIDENCE BETWEEN THE SEXUAL MATURITY OF BRUCHUS AFFINIS (COL. BRUCHIDAE) AND THE APPEARANCE OF ITS EGG-LAYING SUPPORT: PODS OF LATHYRUS SPP. (LEGUMINOSAE)

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In females of **Bruchus affinis** Frölich, termination of reproductive diapause is closely associated with the presence of **Lathyrus sylvestris** and **L. latifolius** L. flowers (Bashar et al., 1985) and young green pods are the egg-laying support. A survey conducted in summer 1985 showed that while appearance of the pods in a **Lathyrus** population is a progressive mechanism, all the **B. affinis** females are reproductively mature and when the first pods appear they massively lay their eggs on them. Consequently, there is a high concentration of eggs on the early coming pods which brings a severe intraspecific larval competition. Moreover, the percentage of seed producing pods is very low at first, may be due to the action of a great number of **B. affinis** larvae. Therefore, this temporal difference between the phenology of the plant and that of the bruchid leads a notable depression effect on the abondance of the phytophagous insect.

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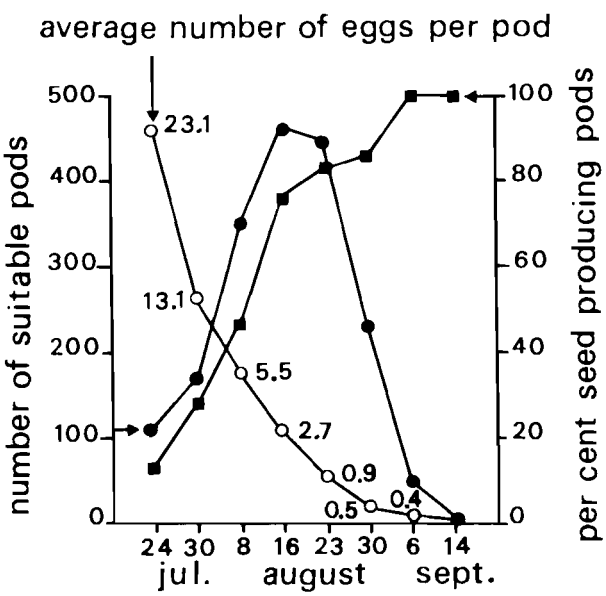

CHEMICAL RESISTANCE OF YAMS TO LEAF CUTTING BY THE ATTINE ANT ACROMYRMEX OCTOSPINOSUS (REICH)

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1. Introduction

The Attini tribe regroups the fungus-growing ants, of which the two major genera, **Atta** and **Acromyrmex**, are serious pests of neotropical agriculture and forestry. In Guadeloupe, we reported the occurrence of a strong specific/varietal resistance in yams (Dioscoreaceae) to attacks by **A. octospinosus** and the involvement of chemical factors extracted from resistant yam leaves (Febvay et al., 1985). We describe here the influence of saponin levels of different yam varieties on ant foraging behavior.

2. Materials and methods

Foraging activity is measured according to Febvay et al. (in press). Three runs are performed for each variety and the average foraging index is used in the following plot. Total saponins are assayed by a modified procedure of that previously used (Febvay et al., 1985). A partial purification scheme of foliar saponins involves a Soxhlet hexane-delipidation and methanol extraction, followed by gel filtration in methanol (Sephadex LH20).

3. Results and discussion

The 27 yam species tested display a wide range of acceptability. The figure 1 shows an inverse relationship between saponin levels and foraging intensity, but not in a clear dose-dependent manner.

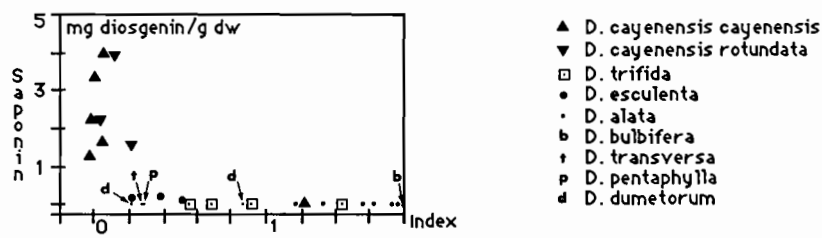

Figure 1. Plot of saponin content of 27 yam species vs foraging index of **A. octospinosus**

In addition, though the methanolic extract retains the foraging - inhibition factor, the LH20 fractionation doesn't allow further purification of this activity. Neither the dioscin-rich LH20 fraction, nor commercial or purified saponins show any inhibitory effect on ant activity, although bearing clear fungal toxicity (decreased nest volume). Therefore, recognition of resitant species doesn't seem directly related to saponins not to an single purifiable factor.

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PHENOLOGY OF QUERCUS SUBER (L.) (FAGALES) AND POPULATION DYNAMICS OF LYMANTRIA DISPAR (LEP. LYMANTRIIDAE) IN MOROCCAN CORK-OAK GROVES

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In the forests of the Atlantic coast of Morocco, populations of **Lymantria dispar** evolve according to two different regimes: - latent regime (i.e. ever-low density, there is no more than 1 gradation over a 60-year period) in some forests; - a recurrent regime (i.e. 5-6 gradations over a 60-year period) in some others. There is some evidence that in the cork-oak there is a partial renewal of foliage in spring in the former-type forests while a complete renewal in the latter. **L. dispar** larvae feed readily upon newly-grown leaves while only reluctantly upon old leaves. This directly effects larval growth which is more difficult on older leaves. As a consequence, the availability of suitable foliage is reduced in the first-type forests resulting in an exceedingly low density further depressed by the role of natural enemies such as braconid wasps which are parasites of caterpillars. In contrast, in the second-type forests the amount of suitable foliable available results in a population explosion that follows the starvation period due to complete defoliation. Moreover, defoliated **Q. suber** trees do not produce a new foliage in the next spring resulting in a phenological asynchrony and in the obligation for caterpillars to feed upon old leaves. This reduces their life expectancy and fertility in adults (Fraval, 1984). The sharp retrogadation is followed by a few-year latency period.

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EFFECTS OF THE HOST PLANT AND THE MALE ON OSCINELLA PUSILLA REPRODUCTION

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1. Introduction

Oscinella pusilla Meig. is a Chloropidae Diptera dependant on certain wild and cultivated grasses (Graminae). Barley and wheat are particularly subject to attacks by larvae of this Diptera.

2. Materials and methods

This study was carried out at 21°C with a 16 hour photoperiod. The males and females were studied separately or in couples, with or without the plant (wheat), during the different periods of their imaginal life.

3. Results

3.1. Role of the host plant. As soon as the first days of imaginal life, the plant attracts the young adults and facilitates copulation. Ovarian maturation is hastened, it is established when copulation takes place (3-4th day). At the same time the female becomes sexually receptive (Gagnepain, 1984). The insect perceives the plant stimulation at a distance (Hamilton et al., 1979) and by contact. Both stimulations are complementary. During the entire reproductive life, the presence of the plant favors the successive copulations (an average of 3) which are necessary for the complete expression of the female reproductive potential (an average of 83 eggs). When the plant is absent, egglaying is practically nil and ovogenesis is considerably slowed down. The mature ovocytes are progressively retained (2 to 3 per ovariole); after 3 weeks of retention 7% of the ovarioles show oosorption in the follicles during vitellogenesis. The female longevity increases.

3.2. Role of the male. Copulation is necessary to the egglaying process, but its stimulatory action on ovogenesis is weak. Without the male but with the plant, mature ovocyte retention is very rarely accompanied by oosorption.

4. Conclusion

In **Oscinella pusilla**, ovogenesis and oviposition are controlled by the presence of the plant. Furthermore, the latter facilitated copulation which is itself necessary for egglaying. This double control increases the egglaying efficiency of this low fertility Diptera.

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TANNIN INHIBITION OF COWPEA BEETLE, CALLOSOBRUCHUS MACULATUS F., LARVAL GUT β-GLUCOSIDASE

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Tannins are polyphenolics known for their ability to precipitate proteins, including enzymes. Goldstein and Spencer (1985) demonstrated tannin inhibition of the plant β-glucosidases which mediate cyanogenesis. The present study investigates the interactive effects of tannin and cyanogenic glycoside for the cowpea beetle.

Feeding studies were done to examine the effects of tannin (quebracho) cyanogenic glycoside (amygdalin), and combinations of the two on beetle development time, survivorship, and viability. Gelatine capsules suitable for oviposition by adult beetles were packed with ground cowpea flour, and combinations of quebracho and amygdalin. Quebracho was found to significantly increase development time and reduce survivorship at 10^{-5}% wt. ($P<0.01$) with 100% mortality occurring at 5% wt. Amygdalin increased development time and reduced survivorship at 0.05% wt. 1 ± 0.63 ($P<0.01$) adults emerged from capsules containing 2% wt. amygdalin (8.6% emergence relative to control). The addition of 10^{-3}% wt. quebracho (37.9% relative to control, $P<0.01$) to capsules containing 2% amygdalin enhanced survivorship with 4.8 ± 1.83 ($P<0.01$) individuals emerging (41.4% relative to control). Progeny from all treatments were viable.

Enzyme assays were done to ascertain the effect of quebracho on larval gut β-glucosidase. Third instar larval guts were dissected, homogenized in pH6 buffer and added to 1M amygdalin and 0–100 mg ml^{-1} tannin in the outer well of a Warburg flask. HCN released from this reaction mixture was trapped by NaOH in the center well. Total HCN was determined after 24 hr. using a modified Lambert colorimetric assay. Quebracho was found to quantitatively inhibit cyanogenesis. The amount of tannin added to the reaction mixture varied inversely with the amount of HCN released.

Tannin was found to mitigate the toxic effects of cyanogenic glycoside in C. **maculatus**. This may be a result of tannin inhibition of larval gut β-glucosidase, which is consistent with the in vitro findings that tannin inhibits cyanogenesis. Many plant defense compounds may rely on enzymic hydrolysis for biological activity - cardiac glycosides, iridoid glycosides, phenolic glycosides, etc. The results presented suggest that in certain situations of herbivory tannins may interfere with the release of the toxic component from any enzymatically mediated defense, thereby reducing the toxic effect.

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WHAT SENSORY ORGANS CONTROL SELECTION BY LEAF-CHEWING CATERPILLARS?

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The roles of olfactory and gustatory (and to some extent, visual) organs in food plant discrimination were examined in larvae of **Manduca sexta** by assaying animals in which various chemosensory organs had been surgically abated. Food choice discrimination was assayed using two-choice leaf disc tests. Three plant species were used for testing: a host, tomato (**Lycopersicon esculentum**); an acceptable non-host, rape (**Brassicae napus**); and an unacceptable non-host, canna (**Canna generalis**). Results show that olfactory and gustatory organs are both used to discriminate the host; the presence of either will result in normal discrimination among the acceptable non-host, but only gustation plays a role in rejecting the unacceptable plant. This rejection can be mediated by a single (unilateral) gustatory sensillum, the medial styloconicum, in animals lacking all other chemosensory organs.

Removal of all known chemosensory organs result in failure to show discriminatory behavior in feeding bioassays if special care is taken to exclude all non-chemosensory stimuli. For example, the normally strongly stimulating hexane extract of tomato leaves, spotted on glass fiber filter paper, will not elicit feeding in totally ablated animals if the assays are run in the dark. This demonstrates that all external chemoreceptors are now identified in **Manduca.**

If these totally chemosensory-ablated animals are assayed in the light, however, we still see some residual discrimination between the tomato extract and the control. We suggest that this may be due to the slight yellow-green hue of the extract. If leaf discs of the different species are assayed (in either light or dark) using totally ablated animals, significant differences in consumption can be seen. We suggest that the differences in mechanical properties and/or hue may be responsible. Thus it is likely that non-chemosensory organs contribute to discrimination if chemosensory organs are lacking.

In summary, the type of chemosensory organs needed for food plant discrimination varies with the plant species sampled. In animals lacking external chemosensory organs, color vision may be used in making feeding choices. However, when chemosensory organs are present, there is little or no visual contribution to food discrimination.

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RESPONSES OF OVIPOSITING ONION FLIES TO AUTHENTIC AND SURROGATE ONIONS

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Recent experiments have shown at ovipositing onion flies (**Delia antiqua**) are very sensitive to stimuli of onion foliage (Harris & Miller, 1984; Harris et al., in press, and references therein). Manipulations using foliar models (surrogates) indicate that onion flies respond to changes in color, shape, size, and chemical cues. Foliar surrogates developed from these investigations compare favorably with authentic onion plants in choice and no-choice bioassays (Harris et al., in press).

Responses to authentic plants and surrogates were examined in greater detail by quantifying behavioral sequences leading up to oviposition (see Harris et al., in press, for procedural details). Timing and locations of behaviors were recorded using a microcomputer. Surrogates consisted of 4 mm diameter glass tubing, painted green and coated with a 0.05% formulation of n-dipropyl disulfide in wax. Onions were in the 3-4 leaf stage, with basal diameters of 3-4 mm and foliar heights of 200-300 mm.

Latencies of pre-ovipositional behaviors on authentic onions tended to be longer but were not significantly different from those on surrogates (one-way ANOVA, $P<0.05$). After alighting, females on onions (n=43) and surrogates (n=20) sat still or groomed for 4.5 and 3 seconds, respectively, before running down the foliage. Extension of the proboscis to foliar surfaces occurred 19 and 6.5 seconds after arrival on onions and surrogate, respectively, and was followed by probing of foliage and soil with the ovipositor 40 and 37.5 seconds later. Insertion of the ovipositor into soil crevices (oviposition) began 117 and 88 seconds after arrival on authentic and surrogate plants, respectively. Ovipositing females repeated this sequence several times before leaving oviposition sites, and generally laid only 1-4 eggs after each sequence. Females on surrogates laid a slightly, but not significantly, higher proportion of the mature eggs in their ovaries (32%) than females on onions (22%), but allotted similar amounts of time to running, mouthparts, and probing behavior. The only significant differences observed in behaviors on authentic and surrogate onions was in time allotted to probing; for each egg laid, females on onions spent more time examining the substrate and foliage with their ovipositors (19 seconds) than females on surrogates (8 seconds).

In conclusion, female onion flies respond similarly to authentic and surrogate onions. Host plant model such as these will be useful tools in both basic and applied research on plant-insect interactions.

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FREE AMINO ACIDS METABOLISM IN TWO WHEAT CULTIVARS INFESTED BY RHOPALOSIPHUM PADI

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The plant of two cultivars of winter wheat, Slavia and Mironovska 808, cultivated on Knop's solution in growth chambers (21 ± 1°C, 60% RH, 7000 Lux, 16L:8D photoperiod) were infested by **Rhopalosiphum padi** L., at the stage of 2 nd leaf (every plants was infested by one apterous female). Control plants without aphids were in the same conditions. At the stage of 5th leaf the aphids were counted and above ground parts and roots of control and infested plants analysed for free amino acids content.

The average number of aphids on Slavia was significantly higher than that on Mironovska 808. The total free amino acid content in above ground parts of infested plants was significantly greater (1.14 time in Mironovska 808, and 1.19 time in Slavia) and the one of roots smaller (0.66 time in Mironovska 808, and 0.51 time in Slavia) than in the control. Significantly different were the concentrations of γ-aminobutyric and glutamic acids. In infested plants the level of γ-aminobutyric acid was increased both in above ground parts (2.83 time in Mironovska 808, 2.40 time in Slavia more than in the control) and in roots of Mironovska 808 (2.39 time more than in the control). By contrast, the conentration of glutamic acid significantly decreased in infested plants of both cultivars (Mironovska 808 0.1, and Slavia 0.5 of the control). Most samples of above ground parts of infested plants contained less valine, leucine, isoleucine, and tyrosine and more of aspartic acid than the control. The concentration of all aminoacids (with exception of γ-aminobutyric acid in Mironovska 808) in roots of infested plants decreased, more in Mironovska 808 than in Slavia.

The aphid infestation evoked deep changes in the nitrogen metabolism of both wheat cultivars. γ-aminobutyric acid accumulation in infested plants is typical for senescent plant tissues. It may influence suitability of a host plant for aphid development. The greater changes of nitrogen metabolism in Mironovska 808 than in Slavia indicates greater sensibility of the former cultivar to aphid infestation.

THE URANIA-OMPHALEA INTERACTION: HOST PLANT SECONDARY CHEMISTRY

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There is a strikingly close larval foodplant association between day-flying Uraniine moths and lianas and trees in the genus **Omphalea** L. (Euphorbiaceae). The pantropical distribution of moths and plants indicates that the relationship predates the separation of the southern continents (Coleman and Monteith, 1981).

The brilliant colouration and conspicuous behaviour of the diurnal Uraniine species suggests toxicity as a defence mechanism. Few insects regularly feed on Central American **Omphalea** species, presumably due to the presence of deterrent secondary compounds in the leaves (Smith, 1983).

As part of a study of the chemical ecology of the interaction, dried leaves of three **Omphalea** species were screened for unusual nitrogenous constituents. Here we report the occurrence of a polyhydroxyalkaloid compound previously unknown in the Euphorbiaceae.

Nitrogenous compounds were investigated by ion exchange chromatrography and high voltage electrophoresis. A weakly basic ninhydrin-yellow compound detected in all plant species was isolated for characterisation from leaves of **O. diandra** L. NMR analysis revealed a five-membered nitrogenous ring bearing two CH_2OH funtional groups. The compound co-chromatographed with an authentic standard of 2R,5R-dihydroxy-3R,4R-dihydroxypyrrolidine (DMDP). DMDP is an analogue of the sugar fructose, differing essentially in the replacement of oxygen by nitrogen.

Polyhydroxyalkaloids resembling monosaccharides have been previously reported from three higher plant families: the Moraceae, the Leguminosae, and the Polygonaceae (Fellows et al., 1986). Many are potent inhibitors of glycosidase activity. Their disruptive effects on the enzyme systems of insects, mammals and microorganisms suggests that these compounds function in plant defence.

The polyhydroxyalkaloid DMDP was isolated from leaves of **O. diandra.** The ability or Uraniine moths to tolerate DMDP in their host plant may be the result of a specialised biochemical/enzymatic adaptation.

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TOXICITY OF ENCELIA (ASTERACEAE) CHROMENES TO PEST INSECTS

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The antihormonal actions of the precocenes (methoxychromenes) have been the subject of considerable investigation (Staal, 1986), but the biological activities of the more widely-distributed acetylchromenes and benzofurans against insects have received little attention to date. We have examined the toxicity of several chromenes ande benzofurans to two pest species, the variegated cutworm (**Peridroma saucia**, Noctuidae) and the migratory grasshopper (**Melanoplus sanguinipes**, Acrididae). The compounds we tested are the dominant natural products occurring in species of **Encelia** and closely related genera, sunflowers common to arid regions of North America. Pure compounds were dissolved in a volatile carrier solvent, and coated onto the inner surfaces of 20 mL glass vials. After removal of the carrier, neonate larvae or nymphs were placed in the vials (with food provided) and survival assessed at 24 - 72 hours (Isman & Proksch, 1985).

Eleven chromenes (seven naturally-occurring) have been tested against **Peridroma**; four of these are relatively insecticidal (Isman et al., 1986a). The most toxic of these is the allatocidin precocene II, with and LD_{50} of 0.67 ug cm^{-2}. Desmethoxyencecalin and encecalin, from **Encelia** species, have LD_{50}'s of 0.98 and 1.15 ug cm^{-2} respectively. Chromene analogues possessing free hydroxyl groups or saturated heterocycles are significantly less toxic. None of the five benzofurans tested are insecticidal at the screening concentration of approximately 5 ug cm^{-2}.

Relative toxicities of selected chromenes to **Melanoplus** are consistent with those in **Peridroma**, although **Melanoplus** is somewhat more sensitive to precocene II and less sensitive to encecalin than is **Peridroma** when differences in live weights are accounted for (Isman et al., 1986b). Toxicity of encecalin to **Peridroma** is antagonized by the benzofuran euparin; toxicity of precocene II is antagonized by euparin in both **Peridroma** and **Melanoplus**. We are currently investigating metabolic and pharmacodynamic differences which may account for these phenomena.

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PHOTOTOXICITY, PHARMACOKINETICS OF ALPHA TERTHIENYL IN SENSITIVE AND RESISTANT HERBIVOROUS INSECTS

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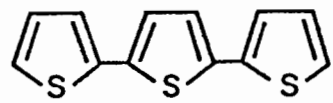
Thiophenes are among the major secondary substances of the plant family Asteraceae. The role of a phototoxic representative of these compounds, α-terthienyl, as part of the chemical defence of composites against 3 species of herbivorous insect, was investigated.



The compound was administered either topically on the dorsal surface of the insect or incorporated in an artificial diet and fed to insects. Alpha-terthienyl was highly phototoxic to the tobacco hornworm, **Manduca sexta** and the cabbage butterfly, **Pieris rapae** (topical LD_{50} for last instar larvae were 10 and 15 ug/g respectively). It was less phototoxic to the European corn borer, **Ostrinia nubilalis** or the tobacco budworm **Heliothis virescens** (LD_{50} 698 and 474 ug/g respectively).

In the feeding studies at 10 and 31 ug/g of α-T in the diet, larval survivorship (% of control) was 100 and 97 for **O. nubilalis** and 70 and 0 for **M. sexta.** Necrotic lesions leading to ecdysis failure and pupal deformities were some of the gross effects of phototoxicity observed in **M. sexta.** In order to investigate the reasons for sensitivity or resistance the pharmacokinetics of ^{3}H-alpha terthienyl prepared by a new exchange process was studied.

In continuous feeding experiments with ^{3}H-αT, after 48 hrs of feeding the % of radiolabel in the body to feces was 59:42 for **M. sexta,** 33:68 for **H. virescens** and 25:75 for **O. nubilalis** respectively. This clearly suggested that **M. sexta** which never encounters composites due to its oligophagous feeding habit was unable to excrete this unfamiliar allelochemical. However **H. virescens** which occasionally feeds on composites, and is notoriously polyphagous, and **O. nubilalis** which frequently feeds on composites containing thiophenes, were able to rapidly excrete this chemical in the feces, preventing lethal concentrations from reaching the cuticle where light mediated toxic interactions may occur. This study suggests that rapid clearance of the phototoxic thiophene is one method by which tolerant insect herbivores deal with this type of allelochemical in host plants.

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RESPONSES OF CENTRAL NEURONES IN THE COLORADO POTATO BEETLE TO GREEN ODOUR COMPONENTS

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Chemoattraction of the Colorado potato beetle, **Leptinotarsa decemlineata** Say, towards its host plant potato, **Solanum tuberosum** L., depends on the composition of the so-called green odour (Visser & De Jong, this volume). The green odour of potato leaves consists of cis-3-hexen-1-ol, cis-3-hexenyl acetate, trans-2-hexenal, trans-2-hexen-1-ol, and 1-hexanol. The perception of an odour blend would involve both the sensitive detection of components, and the perception of blend composition. The olfactory receptors on the beetle's antennae were previously studied (Ma & Visser, 1978; Visser, 1979, 1983). Receptors are tuned to green odour components, and may code for differences in odour blend compositions.

Olfactory antennal receptors are directly connected to central neurones in the antennal lobe in a network of fine arborizations called glomeruli. Intracellular recordings were made from these central neurones in order to study the further processing of antennal information (De Jong & Visser, in prep.). On stimulation of antennal receptors by individual green odour components, central neurones respond with either an increase or a decrease of their spontaneous neural activities. In the antennal lobe of the Colorado potato beetle peripheral receptors converge onto approximately 25 glomeruli, and this causes a 100 to 1000-fold increase in sensitivity. The response spectra of central neurones differ from those of peripheral receptors, and can be classified in two groups. Neurones in group 1 respond to several or all green odour components. Group 2 contains neurones which show specific responses to one green odour component: either 1-hexanol or cis-3-hexenyl acetate. The perception of an odour blend is thought to involve: (a) the sensitive detection of the presence of green odour components, and (b) an evaluation of the ratios between components through the detection of incorrect ratios.

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EFFECT OF DIFFERENT BIOCHEMICAL FACTORS ON THE DEVELOPMENT OF MYZUS PERSICAE (SULZER) ON VARIOUS POTATO CULTURES

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Myzus persicae (Sulzer) acts as an important virus vector in seed crop of potato in Indo-Gangetic plains of India. The permissible limit in seed crop is 20 aphids/100 compound leaves. The aphid multiplication rate depends on many factors including the biochemical composition of a particular culture/variety. Development of **M. persicae** was, therefore, studied on the excised leaves of ten promising potato varieties/culturs at 18 $\pm$ 2°C. The excised foliage were analysed for N, P, K, Cu, Mn,Fe, Zn, Soluble N, total and reducing sugars and total phenols. The mean pre-reproductive period (7.0-9.6 days); reproductive period (4.3-15.5 days); fecundity per female (10.2-34.3) and mean life span (13.3-22.5 days) varied significantly ($P<0.05$) among the different potato cultures. Mean aphid multiplication rate (AMR)/unit time also varied significantly from 1.83 (Cv.K.Badshah) to 3.87 (Cv.K.Sindhuri). The association analysis indicated significantly positive correlation between AMR/unit time and reducing sugars. Such an association was found to be negative with total phenols. Also total sugars exhibited significant positive correlation with reducing sugars, N and K. The partitioning of correlations into direct effect of each component and its indirect contribution through other biochemical traits of the excised foliage revealed that nitrogen had positive direct effect on AMR followed by soluble N, reducing sugars, Fe, P, and K. Its magnitude was highest with total phenols followed by Mn, Zn, and total sugars. Total sugars besides affecting AMR directly also affected indirectly via total phenols and Nitrogen.

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THE IMPORTANCE OF HOLDING ON TO THE HOST PLANT

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Attachment and locomotion on the plant surface have posed a major problem in the evolution of phytophagous insects, and plant diversity is such that specialists are likely to be closely adapted to holding on to their host plants. The study presented here compared the way that two similar and closely related aphid species (**Tuberculoides annulatus** and **Myzocallis schreiberi**) hold on to their host plants (**Quercus robur** and **Q. ilex** respectively).

Q. robur leaves are glabrous but fairly coarsely textured on both surfaces, while **Q. ilex** leaves are smooth and waxy on the abaxial surface and densely pubescent on the adaxial surface.

The mechanism of attachment to the abaxial leaf surfaces was investigated by using video and by taking SEM photographs of live aphids standing on leaves (Stork, 1980).

Both aphid species could walk easily on **Q. robur**, by holding their tarsi flat againts the leaf surface and gripping with spines and terminal claws.

This technique fails on the abaxial surface of **Q. ilex** because the dense mat of trichomes prevents purchase being obtained. However **M. schreiberi** are adapted to overcome this problem: the tarsi are held vertical, and they hold on using flexible spatulate hairs which project between the tarsal claws.

T. annulatus often fall off the adaxial surface of **Q. ilex** while **M. schreiberi** grip satisfactorily. In a bioassay which tested how well the aphids held on to a smooth surface, 63% of the **T. annulatus** fell off while a piece of glass was tipped slowly through 180°. None of the **M. schreiberi** fell off. Measurements of individual dry weights and tarsal lengths show that **M. schreiberi** have relatively longer tarsi ($P<0.05$) than **T. annulatus.** If the size of the structure used for gripping smooth surfaces varies in proportion to tarsal length, then **M. schreiberi** may hold on to smooth surfaces better because they have a larger surface area with which to do so.

These results demonstrate that attachment to the host plant may be a host-specific adaptation in aphids. That one species varied its tarsal position appropriately, and was therefore able to discriminate between physical features of different substrates, suggests that mechanisms underlying attachment to the host plant could also provide a basis for host selection.

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ISOLATION AND IDENTIFICATION OF PHYTOECDYSTEROIDS USING PREPARATIVE HPLC AND 2D-COSY PROTON NMR

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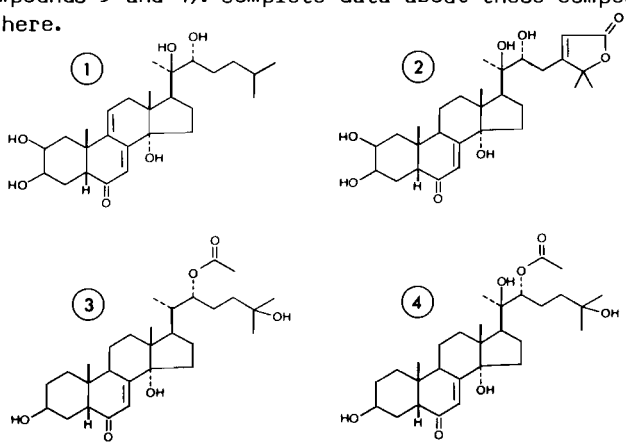
Many plants have been shown to contain substances more or less similar to insect molting hormones (=ecdysteroids). At the present time more than 60 different phytoecdysteroids have been isolated and identified (Horn & Bergamasco, 1985). Such substances are thought to protect plants against (non-adapted) phytophagous insects (Bergamasco & Horn, 1983).

The distribution of phytoecdysteroids among plants is widespread and such substances have been found in ferns, gymnosperms and also angiosperms. Their identification relies on a combination of several techniques including mass spectrometry and NMR and usually needs milligram amounts. The improvement of analytical methods, especially related with the development of evolved two-dimensional proton NMR procedures (Girault & Lafont, 1986 and references therein) allows complete structure elucidation of new compounds with less than 0.1 mg, provided that they are obtained in very pure form, as is the case with preparative silica HPLC. These procedures have been used for the identification of several new ecdysteroids isolated from **Leuzea chartamoides** (compounds 1 and 2) and from **Silene otites** (compounds 3 and 4). Complete data about these compounds will be reported elsewhere.



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SPECIFICITY IN RESPONSES OF FLEA BEETLES (PHYLLOTRETA SPP.) TO FLAVONOIDS

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The investigation is part of a study on the interaction between monophagous beetles and their cruciferous host plants.

In nature, the horseradish flea beetle, **Phyllotreta armoraciae**, utilizes only one host plant, horseradish (**Armoracia lapathifolia**). It has been shown that glucosinolates from horseradish and various non-host crucifers are feeding stimulants for **P. armoraciae** (Nielsen et al., 1979a). Later two flavonol glycosides were identified from horseradish leaves and at least one (kaempferol-3-O-(2-O-β-D-xylosyl-β-D-galactosid) was a potent feeding stimulant (Nielsen et al., 1979b; Larsen et al., 1982). The simultaneous presence of glucosinolates and specific flavonoids could be the cue allowing P. **armoraciae** to distinguish horseradish from other crucifers.

This hypothesis is supported by the following findings:

1. Flavonol-3-O-(2-O-β-D-xylosyl-β-D-galactosides) have not been reported from other crucifers.
2. Most flavonol glycosides with other carbohydrate moieties are not feeding stimulants for the horseradish flea beetle. Rhamnosides are even inhibitory to feeding.
3. Flavonol glycosides from radish appear to be 7-O-rhamnosides. These compounds are inhibitory to the horseradish flea beetle, but some of them are feeding stimulants for the oligophagous species, **P. nemorum**, feeding preferentially on radish.

Conclusion. Flavonol glycosides are important feeding stimulants for **Phyllotreta** species. Beetles are able to discriminate between flavonol glycosides with different carbohydrate patterns. This could be a mechanism for discrimination between host and non-host crucifers.

Acknowledgements

The authors are grateful to Prof. H. Geiger, Prof. J.B. Harborne, Prof. T.J. Mabry, Dr. P.A. Pedersen and Dr. S. Takagi for supplies of flavonol glycosides.

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CORN-EUROPEAN CORN BORER TRICHOGRAMMA RELATIONSHIP: PRELIMINARY STUDY

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Semiochemicals released by host plants cue orientation and selection of pest-insects and their parasitoïds. We studied some aspects of the relationships between corn-European corn borer (E.C.B.) and trichogramma by mean of behavioural and electrophysiological methods.

Field experiments had pointed out that E.C.B. females showed oviposition preferences toward different maize lines in choice conditions. These choices have been reproduced in semi-natural and laboratory conditions demonstrating the role of olfaction. This choice is governed by maize genotype and leaf age.

Electroantennography (E.A.G.) experiments have been performed on isolated female antennae with chemicals identified in maize leaf volatiles: the most stimulating compounds are general occurring volatiles like aliphatic alcohols in C_6 - C_7. However, a great variability of responses was observed and could not be significantly reduced by expressing results versus responses to hexanol (standard compound). A computer program was developped to automatically record and analyse E.A.G. data.

Trichogramma - E.C.B. relationhsips were studied under laboratory conditions to describe egg searching and handling behaviour. Video recording allowed us to quantify succession and duration of the different items of oviposition. On natural host (E.C.B. eggs), the first oviposition is shorter than on rearing host (**Anagasta kuelniella** eggs) and the succession of behavioural sequences is simpler and less variable. On both hosts, these two differences were characteristic of the second oviposition compared to the first one. This could indicate a learning process occurring after a first experience.

Experiments on E.C.B. pointed out the role of olfaction in the choice of host plants even by a so called generalist insect. Plant odours may also be implied in searching behaviour of trichogramma.

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ENEMY-FREE SPACE AND THE EVOLUTION OF HELIOTHIS HOST RELATIONS (LEPIDOPTERA: NOCTUIDAE)

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Heliothis virescens Fab. and **H. subflexa** (Guenee) are nearly indistinguishable sibling species with very different host ranges. **H. virescens** is polyphagous while **H. subflexa** feeds only in **Physalis** spp. (Solanaceae). **H. zea** (Boddie) has a broad host range like **H. virescens**. Experiments to compare host relations were conducted using four plants: tomato (**Lycopersicon esculentum**), cotton (**Gossypium hirsutum**), green bean (**Phaseolus vulgaris**) and groundcherry (**Physalis angulata**).

In rearing experiments to determine relative host suitability, **H. zea** and **H. virescens** grew faster and larger on bean and cotton than on **Physalis** and tomato. **H. zea** did not survive on **Physalis**. **H. subflexa** grew equally well on **Physalis** and cotton even though cotton is a dissimilar non-host.

In food preference tests, both **H. zea** and **H. virescens** larvae preferred cotton or bean over **Physalis** and tomato. Older larvae that had been reared on bean or cotton showed stronger induced preferences than larvae reared on **Physalis** or tomato. **H. subflexa** larvae ate cotton as readily as **Physalis** and feeding on either plant resulted in strong induced preferences.

In oviposition tests, **H. zea** moths preferred cotton over bean and tomato over all others. For **H. virescens**, the larval host influenced preferrence except when tomato was a choice. Tomato was highly preferred. **H. subflexa** oviposited only on **Physalis**

Both **H. zea** and **H. virescens** prefer to oviposit on tomato even though it is less suitable and less preferred by larvae than other hosts. This lack of correlation seems maladaptive. However parasitism by **Microplitis croceipes**, a host specific braconid, was only 10% on tomato compared with 33% on bean in controlled experiments (Mueller, 1983). In nature **Heliothis** larval parasitism by **M. croceipes** can be much higher, up to 100% on spring weed hosts (Mueller & Phillips, 1983) and 70% on cotton. Thus oviposition on tomato may be adaptive because larval survival is better. Enemy-free space may be an important factor in host relations (Lawton & Strong, 1981).

H. subflexa is host specific apparently because of restricted oviposition and not because of a dependence on specific nutrients or feeding stimulants found only in **Physalis**. Specialization on **Physalis** may have occurred because this host produces fruits, and is therefore suitable, for the entire period of **Heliothis** seasonal activity. Specialization on other hosts would require a shortening of seasonal activity to coincide with host reproductive phenology. Specialization on **Physalis** may be selectively advantageous because **Physalis** fruits are enclosed in a loose-fitting calyx that may constitute enemy-free space for **H. subflexa** larvae.

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PRELIMINARY NOTES ON THE USE OF GLASS-FACED BOXES AS A TOOL TO STUDY ROOT/HERBIVORE INTERACTIONS

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The use of glass-faced boxes offers a potentially excellent method for studying interactions between roots and their herbivores. This method, however, has not yet been used in this context.

The box is primarily designed to study interactions betwen root-feeding insects and short-lived herbaceous plants. The proposed box type (5 x 50 x 100 cm high) consists of the following parts: Eternite plate (5 x 50 x 100 cm high), glass plate (0.2 x 50 x 100 cm high), 2 wooden vertical supports (3.5 x 3.5 x 100 cm high, wooden bottom support (3.5 x 3.5 x 44 cm long), conical-shaped, hardened PVC clamps of the type used by electricians, to fix the eternite and glass plate (2 pieces 100 cm long, 1 piece 40 cm long). In addition, both sides of the box were covered with a styropor plate to protect the roots from light and to provide temperature insulation. The boxes were inclined at an angle of 10^{0} from the vertical, with the glass downwards and kept in half shade. An air-dried sand-soil mixture was first sieved through a 1 cm^2 sized mesh, then slightly moistened and filled into the container in successive firmed layers of 10 cm. Uniform sized seeds were placed directly into the sand soil mixture.

To monitor the dynamics of root development, the monthly rooting intensity, i.e. the length of new roots visible per viewing surface, was traced with a waterproof felt-tip pen on an acetate sheet, which was placed directly on the glass plate. Different colours were used for each sample date and the root length increase per sector was later measured by means of an opisometer (rotating wheel) (cf. Böhm, 1979, for a discussion of root parameters and alternative recording methods).

The method is mainly thought to be useful for short term (up to two years), comparatives studies, e.g. in biological weed control pojects involving root herbivores. It permits the study of the impact of different levels of herbivore loads and its interference with additional stress factors, such as soil type, water and nutrient shortage on plant parameters. Assessment of the feeding behaviour of root herbivores and the phenology of their attack, as well as investigations of intra- and interspecific competition of candidate agents (Müller, in prep.) can also be conducted with this method.

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MOLECULAR PARAMETERS INVOLVED IN HONEY BEE OLFACTORY SELECTION OF SUNFLOWER: METHODOLOGICAL APPROACHES

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In sunflower, hybrid seed production strictly depends on entomophilous pollen carryed from male to female lines. Honey bees are the main pollinators of this crop; their foraging behaviour is based on a conditioning process where plant chemicals are mainly involved: during food uptake foragers use to memorize mainly plant aromas, as orientation cues. Thus, in order to analyze sunflower-honey bee relationships leading to sunflower improvement through hybrid seed production, basic studies of volatile stimuli releasing bee attraction have been carried out a different levels (field and laboratory experiments), through combined behavioural and chemical analyses.

Field experiments are carried out under tunnels with honey bee colonies pollinating two pairs of parental lines producing commercial hybrids with different hybrid seed yields. It appears that foragers exhibit a selective behaviour towards the pair of lines with a low level of seed production, while they visit randomly the pair of lines with a high seed yield.

Extracts obtained from aromas headspace trapping of each line, are chemically analyzed by gas chromatography. Among hundreds of compounds separated, statistical comparison of chromatographic profiles points out qualitative and quantitative differences restricted to about 10% of the detected compounds. Thus, foragers are likely to discriminate plant genotypes using slight differences among complex volatile blends.

Such data are suported by further experiments combining chemical analysis (by coupled GC-MS) and fractionning, and a biological test using an artificial flower device in controlled conditions (Pham-Delegue et al., 1986): dichloromethane extracts of a blend of sunflower genotypes is submitted to honey bees as a conditioning scent. Then, fractions of the whole blend are tested versus the conditioning blend: fractions confused with the conditioning blend are considered as active fractions of sunflower aroma. Behavioural responses indicate that honey bees are able to identify plant aroma from a limited fraction of the whole blend (10%). These data allow to point out honey bees ability to identify a complex volatile information from a "limited aromatic pattern".

Currently, a method of coupling simultaneously GC with animal detection (AD) is set up in order to precise the biological activity of volatile compounds. GC is therefore used as an olfactory stimulant which separates compounds from natural blends, at known concentration. Different types of biological responses triggered by olfactory cues may be recorded from bees, as electrophysiological responses (classical electro-antennogram and/or unit recordings) and behavioural responses (through a conditioned reflex behaviour of proboscis extention).

This work should lead to define some molecular parameters involved in honey bee olfactory discrimination among plant genotypes, likely to become tools for plant improvement through entomophilous pollination.

LEARNING OF HOST ACCEPTANCE IN THE APPLE MAGGOT FLY, RHAGOLETIS POMONELLA: THE ROLE OF FRUIT SIZE

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Prior adult experience with host apples or hawthorn fruits alters the propensity of a female apple maggot fly, **Rhagoletis pomonella** (Family Tephritidae), to attempt oviposition into these fruits. In laboratory studies, we have attempted to characterize the stimuli involved in learning of host acceptance by the apple maggot fly. A previous study (Papaj & Prokopy, 1986) established that fruit size and surface chemistry were stimuli towards which behavioral responses were altered by prior experience with real fruit of particular sizes. In the following laboratory study, we examined whether behavioral responses to fruit size could also be altered by prior experience with artificial fruit models of particular sizes.

Adult female apple maggot flies were permitted to oviposit for 7 days into either artificial fruit models similar in size to hawthorn fruit (15 mm diam.), models similar in size to apples (65 mm diam.), or no models at all. Artificial models consisted of spheres of agar and crushed apple fruit coated with a thin layer or red ceresin wax. After 7 days, all flies were tested for their propensity to accept models of each size. Acceptance was recorded if the fly attempted to oviposit in (i.e., bore into) the model. Rejection was recorded if the fly few from the model or remained on the model for 5 minutes without boring.

Flies exposed to hawthorn-sized models (N=32) bored into apple-sized models much less often than did flies exposed to no models (i.e., naive flies; N=43; 28% vs. 64%, G-test, $P<0.01$). The same flies bored slightly more often into hawthorn-sized models than did naive flies (94% vs. 83%, G-test, n.s.). Flies exposed to apple-sized models (N=33) bored slightly more often into apple-sized models than did naive flies (71% vs. 64%, G-test, n.s.). The same flies also bored slightly less often into hawthorn-sized models than did naive flies (74% vs. 83%, G-test, n.s.). A three-way G-test on the independence of female response, exposure regime and test fruit indicated that the overall acceptance of test model of each size depended strongly on the size of the model with which females were experienced (Response x Test x Experience Effect, $G=48.45$, $P<0.0001$).

We conclude that experience with an artificial oviposition substrate of a particular size can reduce a female apple magot fly's propensity to accept substrates of a different size. Since the models to which females were exposed differed only in size, prior experience with size stimuli alone and no other fruit stimuli is apparently sufficient to alter future oviposition response to fruit size.

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A MODEL OF HOST PLANT CHANGE OF ZABROTES SUBFASCIATUS BOH. (COLEOPTERA: BRUCHIDAE) IN A TRADITIONAL BEAN CROPPING SYSTEM IN COSTA RICA

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Field studies in a traditional bean cropping system ("Frijol tapado") showed that an average of 8% of the **Phaseolus vulgaris** pods harvested were attacked by **Zabrotes subfasciatus.** Levels of attack observed on wild **Phaseolus lunatus** vines also present in the agro-ecosystem were comparable (x=9%).

Data obtained on the insect's reproductive biology, a survey of its host plant records, the particular attention paid to adaptations likely to restrict its host plant range (physiological, morphological, ecological) and field observations all demonstrated the extreme host specificity of **Z. subfasciatus** in the local community context.

A model of host plant switching by this Bruchid in the Costa Rican region studied was proposed after testing its underlying assumptions. The model predicts that the level of bean crop contamination could be reduced by controlling this insect's wild host plant (**P. lunatus**) around P. **vulgaris** fields and bean storage sites.

Models embodying knowledge on the natural history of bruchids, together with a biogeographical point of view, can help to organise one's thoughts when interpreting patterns of grain contamination and making policies relevant for the design of safe pest control strategies compatible with a self-reliant rural development.

STRUCTURE AND FUNCTION OF THE PALPI OF COLORADO POTATO BEETLE, LEPTINOTARSA DECEMLINEATA SAY

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The Colorado potato beetle, **Leptinotarsa decemlineata**, is an oligophagous pest restricted in its choice of food plants to the family Solanaceae. Behavioral studies with adult beetles indicate that prior to feeding, the maxillary palpi tap the leaf surface while the labial palpi are more or less in continuous contact, thereby suggesting that the palpi help in sensory identification of the host plant by recognizing leaf surface waxes, which are specific to a particular plant species.

Based on SEM and TEM studies, there are about 185 sensilla on the apex of the maxillary palpi and 65 sensilla on the labial palpi. Both contact and olfactory sensilla are seen. Bioassays conducted with adult beetles using leaf discs and millipore filter discs clearly indicate the importance of palpi in host plant selection. Waxing the palp inhibits sensory input and results in longer time durations to take the firts bite after initial contact. This is also evidenced by the lower amounts of leaf disc consumed by beetles with their palpi waxed. When exposed separately to six solanaceous species, potato offered least resistance to feeding in having lower values for time taken to take the first bite. Other species in order of preference were **S. elaegnifolium, Datura stromonium, S. nigrum, Lycopersicon esculentum** and **Nicotiana tabacum.** Interestingly, the order of preference was similar when surface waxes extracted from these six species were given separately thereby suggesting the leaf surface waxes probably contain the stimuli important in host acceptance.

Detailed chemical analysis was done on leaf surface waxes of potato using FTIR, GC and MS. Of the different fractions tested, it appears that the chloroform fraction, which essentially consist of primary and secondary alcohols, contains the stimulating compounds. The distribution of various types of sensilla on the apical dome of the maxillary and labial palpi of **L. decemlineata** suggest that olfactory sensilla are predominant. The rapid movements of the palpi probably serve to increase ventilation around the palpi ensuring a high resolution of incoming stimuli. The percentage of gustatory sensilla, though low, seems appropriate to explain the behavioral sequence whereby the palpi tap the leaf surface.

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XYLEM INGESTION BY APHIDS

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The d.c. based method for recording aphid feeding behaviour results in the prodution of the "electrical penetration graph" (EPG) (Tjallingii, 1985). This consists of a number of waveforms which can be characterised and distinguished by their frequency and amplitude. The usefulness of this technique depends on the correlation of waveforms with stylet activities in plants. Two electrical patterns in particular, patterns E (pd) and G have been strongly correlated with uptake from artificial diets, suggesting that their occurrence on plants is related to ingestion.

The pattern G waveform shows two distinct electrical components, one of which is of emf origin (i.e. originate within the plant and aphid system) and the other is the result of changes in electrical resistance. Three aphid species, **Metopolophium dirhodum, Rhopalosiphum padi** and **Acyrthosipon pisum** all showed significant increases in the occurrence of pattern G after a 24h. period of starvation prior to recording, with it constituting up to 30% of the penetration time. With another species, **Aphis fabae**, aphids were starved for either 24 or 48h. prior to recording and significant increases in the occurrence and duration of pattern G were found with each increase in starvation time. These increases were directly related to the amount of water lost by evaporation, as measured by weighing, over the same period. Aphids were also starved for 24h. in low humidity by confining them in a dish containing silica gel. After this treatment the proportion of pattern G in recordings was as high as that from aphids which had been starved for 48h., suggesting that loss of water was an important factor in determining its occurrence.

Stylets of **A. fabae** were severed during pattern G and examination of thin sections with the electron microscope revealed the stylet tips located within a xylem vessel (see plate, tips are arrowed). This suggests that pattern G is indicative of the uptake of water.

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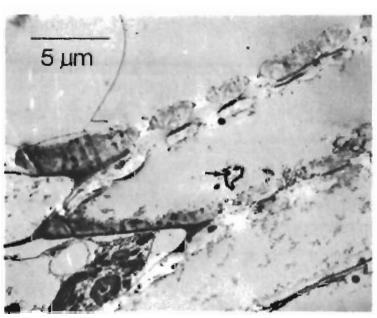

OVIPOSITION DETERRING PHEROMONE OF RHAGOLETIS CERASI: ISOLATION AND IDENTIFICATION USING THE ODP RECEPTOR CELL AS DETECTOR

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The European cherry fruit fly (**Rhagoletis cerasi** L.) deposits a pheromone trail on the fruits by dragging its ovipositor after oviposition. This oviposition deterring pheromone (ODP) inhibits subsequent oviposition attempts by other females of the same species.

A contact chemoreceptor cell with a high specificity and sensitivity for the water-soluble ODP has been identified in the tarsal D-sensilla of male and female flies. The spike (nerve impulse) activity of this ODP-best receptor cell was found to be correlated with behavioural assays (oviposition deterrence) of pheromone extracts. Since for the recordings from ODP-best cells a thousand times less material was necessary to obtain reliable results electrophysiological measurements were the preferred bioassay to detect the ODP acitivity in different fractions of raw extracts.

Using electrophysiological recordings ODP activity was identified in the faeces of females which were older than 3 days. Younger females and males of all ages produced faeces without significant (solution of one faecal droplet) stimulatory activity.

The ODP was isolated from collected female faeces and identified by Hurter et al. (1986). It proved to be as active as the ODP raw extract both in the behavioural and electrophysiological assays (Boller et al., 1986). In the inactive male faeces no or a hundred times less of the compound could be idendified. The structure suggested for the molecule is N(15 (β-glucopyranosyl) -oxy-8-hydroxypalmitoyl) -taurine. The corresponding synthesis is in progress and will in the near future hopefully allow us to verify the proposed structure and to establish the configurations of C-8 and C-15 of the fatty acid constituent.

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INFLUENCE OF LIGNIN EXTRACTED FROM THE TEGUMENT OF PHASEOLUS VULGARIS SEEDS ON THE POST-EMBRYONIC DEVELOPMENT OF ACANTHOSCELIDES OBTECTUS SAY, (COL. – BRUCHIDAE)

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The larvae of **A. obtectus** may be developed in an artificial diet obtained by the compression of the pulverized cotyledons of **Ph. vulgaris**. The introduction of the pulverized tegument of the seeds into this nutrient media prevents the development of most of the larvae (Stamopoulos & Huignard, 1980; Stamopoulos, 1980).

This paper reveals that the lignin extracted from the tegument of the seeds of **Ph. vulgaris** (white var.) could be the substance responsible for this observed tocixity. In fact, the introduction of lignin into the artificial diet of the insect, provokes a much higher larval mortality. The calculated LC_{50} for this substance (Busvine, 1957) is in the order of 0.26% while the LC_{50} for the pulverized tegument prior to extraction is in the order of 2.6%.

It is therefore possible that lignin is the principal "barrier" or the seeds against the larvae of **A. obtectus** and that it plays a decisive role in the biology and behavior of this insect.

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METHODOLOGICAL APPROACH TO IDENTIFY THE CHEMICAL ATTRACTANTS FOR THE GRAPE MOTH LOBESIA BOTRANA SCHIFF

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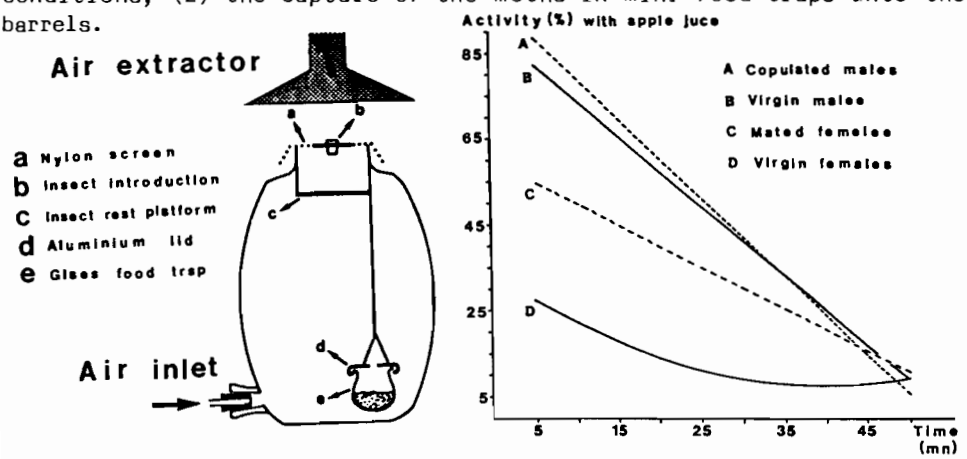
1. Introduction

The ecological studies of insect attractants implicate the use of an efficient biological test. The authors describe a new olfactometer device allowing the screening if volatile compounds.

2. Materials and methods

In this system, a 10 l glass barrel, the moths are able to fly in a 0.5 m/s air flow. It is possible to record two biological responses of insects: (1) the flight activity during a one hour dusk in laboratory conditions; (2) the capture of the moths in mini food traps into the barrels.



3. Results

Before the application this device on extracts of **Vitis vinifera** we tested it with apple juce known to be effective on **Lobesia botrana** moths.

Without any substance, the general activity of the moths is decreasing during the one hour dusk, but the initial level of this activity is depending of the sexual state of moths. First are the virgin and copulated males then the mated females and at least the virgin females. With apple juce (at the same dilution, 20% as in food trapping) the activity level is significantly increased only for copulated males and mated females.

The captures are also depending of the sexual state of insects. They are identical for the males (virgin: 40.5%, copulated: 45.5%) but are different for the females (virgin: 16.5%; mated: 64.5%).

The study of the relationship activity-capture is confirming the olfactive motivation of the mated female behaviour in this experimental device.

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BIOCHEMICAL MECHANISMS OF PEST RESISTANCE IN PASTURE LEGUMES

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Several pasture legumes, including **Lotus pedunculatus** and bean (**Phaseolus vulgaris**) are resistant to larvae of the root-feeding scarab **Costelytra zealandica**. Bioassays have shown that both feeding deterrents and toxins are implicated. Larvae are deterred from feeding by isoflavonoid constituents of the roots (Russell et al., 1978; Sutherland et al:., 1980; Lane et al., 1986). The most active compounds are those taht have also been recognized as phytoalexins in the foliage of plants (for example, vestitol in **Lotus** and phaseollin in bean). The feeding deterrent activity of these compounds is highly structure dependent and relates to their stereochemistry (Lane et al., 1985).

Besides isoflavonoids, the resistant plant **Lotus pedunculatus** contains high yields of glucose nitropropionic esters which are both feeding deterrent and toxic at the concentrations (0.2% ww) found in the root (Hutchins et al., 1984). The nitro esters do not have a particularly high biological activity but they make a substantial contribution to the total feeding deterrent activity of the plant because of their high concentration. By contrast, the isoflavonoids are very active and account for a significant part of the feeding deterrent activity in spite of their low yield (ca. 10 ppm).

Quantitative studies of the relative roles of these non-preference and antibiotic resistance mechanisms of **Lotus** and bean, and of the distinct classes of compounds responsible for each, provides direction for the genetic manipulation of these legumes in an attempt to develop a pest-resistant white clover (**Trifolium repens**).

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ANTIXENOTIC AND ANTIBIOTIC EFFECTS OF SECONDARY PLANT SUBSTANCES IN ARTIFICIAL SEEDS, ON THE DRY BEAN WEEVIL, ACANTHOSCELIDES OBTECTUS SAY (COL., BRUCHIDAE)

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The ability of **A. obtectus** to behaviourally and developmentally overcome allelochemicals not indigenous to its host plants has been investigated.

Bean powder-, potato starch- and water-based pilules were prepared containing 0.16, 1.65 and 8.1% w/w of any of 43 compounds belonging to different chemical classes: alkaloids, phenols, glycosides, organic acids, and the non-protein amino acid canavanine.

Oviposition responses were tested in no-choice (only one kind of pilule offered for 3 females per replicate) and in multichoice situations (43-46 stimuli presented to a population of females). For evaluation of the latter the egg-count data were directly submitted to cluster analysis.

For measuring the ability of the species to develop in pilules holding chemicals L_1s (= eggs just before hatching) were put onto the artificial seeds. Mortalities and the time elapsed till adult emergence were determined.

No correlation seemed to exist between adult oviposition preference for and larval ability to develop in substrates containing any given compound investigated.

In multichoice tests, neonate larvae showed high sensitivity to alkaloids (Janzen et al., 1977). This may reflect the likely importance what these compounds have in host specificity.

In no choice tests, ovipositional responses varied widely and frequently not in accordance with concentrations. In multichoice tests, a constancy in egg-laying responses, relative to concentrations, did exists.

A. obtectus, albeit poorly, is able to develop in pilules containing ca. 2% L-canavanine. Although, this compound is not characteristic of the tribe of Phaseoleae (Lackey, 1977), the following results may suggest the special importance of toxic amino acids in the life of this bruchids: a) the larvae "handled" a relatively high concentration, and b) regardless to concentrations, canavanine was consequently separated from all other compounds by the cluster analysis.

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POLLINATION OF ZYGOGYNUM (WINTERACEAE) BY SABATINCA (MICROPTERIGIDAE)

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The genus **Zygogynum** (Winteraceae) comprised of seven species of small trees (to 8 m) is endemic to New Caledonia. The flowers of at least two species (Z. **baillonii** and Z. **bicolor**) are pollinated primarily by a moth, **Sabatinca** (Micropterigidae). The plant family and the micropterigid moths have a fossil record extending to the Early Cretaceous. The interaction of the two organisms is of particular importance as this is the first report of a primitive angiosperm being pollinated by a micropterigid.

Few flowers are open on a given day with the functional life span of an individual flower covering two days. The male and female moths, an undescribed species of **Sabatinca**, congregate on opening buds and flowers in numbers up to 80 or more. The insects rapidly crawl over the flower, twigs and leaves in the general area of the flower. The moths mate on the flowers and the foral fragrance apparently acts as the primary attractant. The floral odor must act as an assemblying scent that attracts male and female moths. The low number of flowers presented on the trees at any one time enhances the process by concentrating the insects on few flowers. The female-phase flower (first day) emits a strong fragrance, offers no food and closes at the end of the day. The odor recommences during the male-phase (second day) at which time pollen is extruded through terminal slits and hangs in strands from the stamens. The lipid coat serves as a food for the moths and encourages successful attachment of the tetrads to the insects body (wings, legs and lower abdomen become coated). If the moths visit a female-phase flower next, they will crawl over the stigmatic crests searching for pollen or mates and thus pollinate the flower. The floral odors of both species of **Zygogynum** contain a large number of acetate compounds (ethyl acetate, propyl acetate, etc.). The two species differ in quantity of individual chemicals as well as presence with Z. **baillonii** very rich in 2-methyl-butyl-acetate. Most of the chemical compounds are poisons to most insects. It is suggested that floral odors may have been initially derived from chemicals present as secondary compounds in leaves.

The use of flowers as mating and feeding stations appears to be of general occurrence in the primitive angiosperms (particularly for beetle pollinated species). This mode of pollination in which flowers function as mating and feeding stations with floral odors acting as the primary attractant may have been common in the early evolution of flowering plants.

OVIPOSITION AND FOOD-PLANT SELECTION BY THE CINNABAR MOTH, THE EFFECTS OF PROTEIN NITROGEN AND SUGARS

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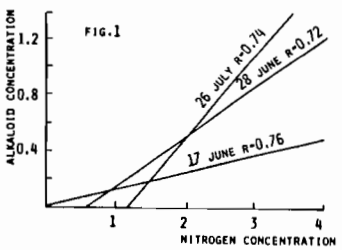

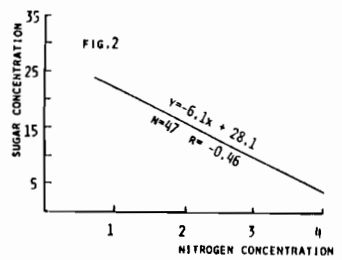

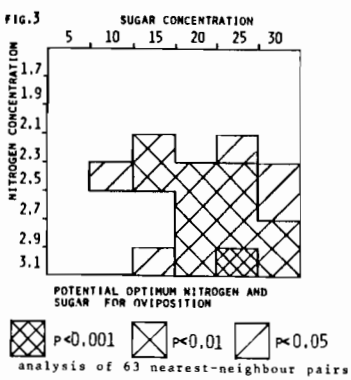

In a growing number of examples, oviposition or food selection does not simply follow a positive relation with nitrogen concentration. Oviposition by the Cinnabar moth only increases with protein nitrogen (% dry weight) of Ragwort till a certain level, and follows an optimum curve (van der Meijden et al., 1984).

This may be due to protein nitrogen itself, to the composition of amino acids, or to an unfavourable factor associated with a high protein-nitrogen concentration. There are at least two associated factors in this animal-plant interaction: alkaloids are positively correlated with protein nitrogen (Fig. 1), water-soluble carbohydrates (sugars) are negatively correlated (Fig. 2). By means of artificial addition on the leaf surface, the separate effects of nitrogen and alkaloids on oviposition and larval success were studied.

Addition (+ 4%) of a natural mixture of amino acids had significant **negative** effects on oviposition and larval growth.

Addition (+ 0.3%) of a natural mixture of alkaloids had no effect.

Selection for oviposition in relation to the concentration of sugars and protein nitrogen was studied on pairs of nearest neighbour plants in the field. In 43 out of 63 pairs, plants with the highest concentration of sugars and protein together were selected (X^2 = 8.40, $P<0.01$). A nearest-neighbour analysis (see van der Meijden et al., 1984) shows that plants poor in nitrogen and in sugars are avoided as well as plants that are rich in only one of these substances (Fig. 3). This can explain the non-linear relation between oviposition and nitrogen when studied separate from sugars.

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ENERGETIC EFFICIENCY IN PHYTOPHAGOUS INSECTS: ITS MEASUREMENT AND VARIATION

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1. Introduction

In insect-plant research, measures of the energetic efficiency with which insect growth takes place are frequently employed as an indicator of host plant suitability or food quality, e.g. Waldbauer's "Efficiency of Conversion of Digested food" ("ECD") (Waldbauer, 1968; Slansky, 1985). Analysis of literature data reveals that among the nutritional indices in common use, the ECD parameter shows the highest degree of variation. Several methodological problems have been associated with the calculation and balancing of energy budgets in phytophagous insects (Axelsson & Agren, 1979; Wightman, 1981).

2. Method

A sensitive differential gas-analysis system is presented which allows automatic, continuous and chronic measurement of both oxygen and carbon dioxide concentrations in a flowing gas stream that passes through a respiration chamber with undisturbed feeding **Pieris** caterpillars during their last instar. Combined with the usual gravimetric determination of dry matter budgets, calculation of energetic efficiency could also be performed independent from the estimate for food intake and both procedures can be compared.

3. Results and conclusions

The variations in energetic efficiency derived from the respiratory metabolic technique presented are considerably smaller than resulting from the gravimetric approach. Metabolic intensity and -efficiency appeared to be rather fixed in the different dietary situations studied. it is argued that variations reported in the literature on this subject are produced by invalid assumptions and random as well as systematic errors inherent in gravimetric budget calculations and do not reliably reflect the relevant physiological variation sought for.

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SPATIAL AND TEMPORAL DISTRIBUTION OF CRYPTIC AND APOSEMATIC CHRYSOMELIDS ON THEIR HOST PLANTS

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We investigated the influence of the predictability and abundance of host plants on trophic specialisation and defensive strategies of some of their herbivorous insects. For this, we compare 3 sympatric chysomelid species: **Cassida viridis** (L.), **Chrysolina polit**a (Motsch.) and **Chrysolina** (Dlochrysa) **fastuosa** (Motsch.), feeding on Labiatae in damp biotopes.

C. viridis was found on 5 host plants (**Mentha aquatica, Stachys sylvatica, Galeopsis tetrahit, Lycopus europaeus** and **Glechoma hederaceae**). It is cryptic and its defense is purely mechanical. **Ch. polita** has 3 host plants (**Mentha aquatica, Lycopus europaeus, Glechoma hederacea**). It is visible and chemically protected by cardiac glycosides. **Ch. fastuosa** has 2 host plants (**Galeopsis tetrahit** and **Lamium album**). It may be considered as a "temporal monophage", because it shifts from one to the other plant according to their availability. it is aposematic and chemically protected as **Ch. polita**

Field data were collected on the insects, their behaviour, the plant on which they were observed, the neighbouring vegetation and abiotic conditions. The results were analysed by correspondance analysis. From this analysis, significant parameters were extracted and the way they affect each species was then compared.

Our results suggest that the niche of the 3 leaf beetle species studied is not only defined by their host plants but also by different characteristics of the individual plants and the behaviour of the insects. For the adults, the following patterns emerge from our analysis:

- aposematic insects choose the less predictable biotopes and are more closely associated with them than cryptic ones.
- each species choose a height in the vegetation making it more or less conspicuous: **Ch. fastuosa** prefers the top of plants and **C. viridis** is the species found the deepest in the vegetation.
- aposematic insects are present in more even number during the season.
- aposematic insects are more aggregated than cryptic ones.

The cryptic **Cassida viridis** and the aposematic **Chrysolina fastuosa** are the most different, whereas **Chrysolina polita** is intermediate.

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GEOGRAPHIC VARIATION IN THE LIFE CYCLE OF APHIDS

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We have discovered that the aphid, **Hormaphis hamamelidis** which makes galls on leaves of witch-hazel, **Hamamelis virginiana**, is monoecious on witch-hazel at high elevations in the Blue Ridge and Shenandoah Mountains of Virginia, USA, but is heteroecious on witch-hazel and river birch, **Betula nigra**, in the Potomac River Valley where its life cycle was accurately described 85 years ago. This case is unusual in that two extremely divergent life cycles appear to occur within one species.

In the Potomac River Valley, alate aphids emerge from galls in June and migrate to river birch to deposit asexual offspring. Fall migrants return to witch-hazel to produce the final sexual generation. In contrast, at high elevations alates from galls are non-migratory and produce the sexual generation directly onto witch-hazel. In this strikingly abbreviated life cycle sexual reproduction occurs in the 3rd rather than the 7th generation as in the lowlands.

Extensive literature documents the importance of environmental factors in aphid development and reproduction, particularly on the timing of sexual reproduction. Therefore, we have performed reciprocal transplantation experiments to determine the genetic and physiological basis of the geographic variation in life history. Overwintering eggs from Virginia mountain sites and a Potomac River Valley site were transplanted between localities, and the reproductive morphology of alates from galls challenged to the two host plants was analyzed.

Preliminary results from March, 1986 transplants suggest that the life cycles of the two variants are largely under genetic control. The development of transplants was accelerated in the lowlands and retarded in the highlands when compared to sham-transplanted controls. However, the reproductive mode of gall alates was not plastic: alates from low-to-high transplants produced asexual offspring; high-to-low transplant alates bore sexual offspring.

In the future, transplants will be performed in the fall in order for developing eggs to experience more prolonged climatic differences. We will also examine the reproductive compatibility and morphology of the two forms to determine whether we have a new species.

Our continuing results will bear directly on the questions of ecological plasticity and the evolutionary basis of developmental and ecological diversity in aphids.

EFFECTS OF HOST PLANT COMPONENT ON STEM BORER LARVAE ESPECIALLY CHILO PARTELLUS SWINHOE: A BEHAVIOURAL AND ELECTROPHYSIOLOGICAL STUDY

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Chemical factors acting at the sensory level to influence stemborer behaviour to susceptible and resistant host plant varieties are least understood. Polar and non-polar extracts from the above varieties are employed to investigate the maxillary taste sensilla responses of **Chilo partellus, Eldana saccharina** and other pests of sorghum and maize. An in vitro behavioural feeding technique to bioassay host plant has been developed.

Evidence based on dose response curves indicate that the maxillary taste styloconica sensilla of **Chilo**, unlike those of **Eldana**, detect differences between host plant extracts. Aqueous extracts from the resistant variety evoked high impulse frequency in the lateral sensilla whereas similar extracts from susceptible variety exerted more influence on the medial sensilla.

Aqueous extracts from a susceptible variety contributed to significantly higher feeding activity and weight gains than all the other extract components tested. On the other hand, the chloroform methanol extracts from susceptible and resistant varieties were equally effective in causing the reverse effect. It has been observed that the major difference between a susceptible and a resistant maize variety lies in the internodes. The continuing studies in close collaboration with chemists are designed to isolate and identify chemical components responsible for the reported differences.

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HESSIAN FLY, MAYETIOLA DESTRUCTOR (SAY) (DIPTERA: CECIDOMYIIDAE), INDUCED CHANGES IN 'WINOKA' WHEAT

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Larval Hessian flies, **Mayetiola destructor** (Say), damage wheat in the autumn by feeding on stems near the crown, depleting nutritive reserves and causing a profound change in the physiology of the plant by stunting it. Spring feeding occurs at the stem nodes, and may cause a loss in grain weight and quality often with "lodging", due to mechanical stem injury. Crown fructans typically increase in wheat as temperatures decline in autumn. Wellso et al. (1986) previously showed that one and four or more Hessian fly larvae per stem depleted 'Winoka' wheat crown sugars by 21 and 50%, respectively, resulting in a concurrent decrease in cold hardiness when compared with control seedlings.

In this study, 'Winoka' wheat seedlings were held at 15.6°C for five weeks (except for a five-day infestation period at 21.1°C) or an additional three weeks at 1.67°C that simulated cooler prewinter conditions. The plant weight above the ground the number of stems, crown weight, and root weight were compared for both date groups between control seedlings and those seedlings infested with one to three large green-gut larvae per stem.

The studies showed that the fly effect was significantly more important than the date effect. In addition, little plant recovery was noted for infested plants between the two date groups.

The overall effects noted were: a 65% reduction in crown weight for both date groups; a 65% (five-week) and 60% (eight-week) reduction in plant weight; a 48% reduction in the number of stems for both date groups; and a 27% (five-week) and 21% (eight-week) reduction in root weights. Little recovery from Hessian fly damage was noted between the fifth and eighth week tests; however, as in previous studies, the fly was shown to be very damaging to wheat.

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RESPONSES OF PERSIMMON TREES TO PERIODICAL CICADA OVIPOSITION DAMAGE

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1. Introduction

Periodical cicadas (Homoptera: Cicadidae: **Magicicada** spp.) emerge synchronously every 13 or 17 years in densities that may exceed 4 million per hectare. Cicadas lay eggs in small twigs (3 to 11 mm diameter) of woody plants, and each female cicada may make hundreds of eggnests that contain up to 25 eggs each, which produce large wounds in many trees. In northwest Arkansas, U.S.A., 13-year periodical cicadas emerged in May-June, 1985. Cicadas frequently used persimmon trees (Ebenaceae: **Diospyros virginiana**) as oviposition hosts. Many persimmon trees secreted gum in response to oviposition damage by periodical cicadas. I used this system to test hypotheses concerning costs and benefits of gummosis to trees.

2. Methods

Twigs of 44 trees were measured and eggnests counted to determine eggnest density (eggnests/cm shoot length). At the end of the season, new shoot growth was measured on 44 trees to determine growth after damage. After eggs hatched, 10-25% of eggnests on 22 randomly-selected trees were collected. Eggnests were dissected, gummosis of eggnests and proportion of dead eggs encased by gum were recorded. To test effects of damage for herbivorous insects, foliage from trees damaged by cicadas in June, 1985, and foliage from undamaged trees were fed to fall webworms (Lasiocampidae: **Hyphantria cunea**), a generalist lepidopteran herbivore of persimmon, in July. One hundred larvae were fed each type of food in the laboratory and pupal weights were compared.

3. Results and discussion

Eggnest desities on study trees ranged from 0-44 eggnests/100 cm shoot (0-158 eggnests/tree). I dissected 170 eggnests and examined 2899 eggs for gummosis. Proportion of eggnests with gum increased as eggnest density increased. Proportion of dead eggs encased in gum increased as eggnest density increased, effectively reducing the hatching success of cicada nymphs. New shoot growth generally decreased as number of eggnests per tree increased. However, when only values from trees with eggnest densities greater than 5/100 cm were considered (n=37), that relationship was highly significant, reflecting the apparent cost of wounding by cicadas and/or gum production. Foliage quality of cicada-damaged trees was not apparently different than that of nearby trees without cicada oviposition damage. Each sex of fall webworm pupated at similar times, sizes, and survival rates, indicating no generalized, long-term antiherbivore response was induced. Since periodical cicadas emerge only in 13-year intervals, evolution of a specific response to cicada damage is unlikely. Rather, gum secretion appeared to be a generalized response to wounding. This research was funded by N.S.F. BSR 84-08090 to K.G. Smith, D.A. James, and F.M. Stephen.

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THE INFLUENCE OF SURFACE CHEMICALS OF SORGHUM ON THE BEHAVIOUR OF THE STEMBORER CHILO PARTELLUS (SWINHOE)

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Field observations in India of newly hatched larvae of **Chilo partellus** on resistant (IS2205) and susceptible (IS1151 and CSH1) cultivars of sorghum indicated, in addition to physical plant characteristics that reduce successful establishment, a resistance mechanism based on the disorienting effects of chemicals on the plant surface. Larvae crawl up from the oviposition site near the base of the plant to the whorl where they feed. The primary stimulus is positive phototaxis, and on IS2205 only, a behaviour patterns was frequently observed which was characterised by hesitation, circling and stopping completely for up to several minutes, accompanied by raising and side to side motion of the head and upper abdomen in a 'searching' movement.

Surface extracts in chloroform were made form the three cultivars. In bioassays the 'searching' behaviour described above was observed only as larvae crowled towards the light over glass plates coated with extracts of IS2205. larvae were disoriented when climbing up plastic rods coated with IS2205 extract, whereas they climbed easily on extracts from the susceptible cultivars.

Analysis of the crude extracts by GC showed similar spectra for all three cultivars, with the exception of a consistent concentration difference in one peak which co-eluted with the C_{32} n-alkane. This peak was more than twice as intense in the susceptible cultivars as in IS2205. It is suggested that, as they crawl over the plant surface the larvae receive chemical cues from which they identify a suitable host plant, and these reinforce their innate phototaxis. If any of the cues is missing, or not sufficiently strong, then the insect is disoriented.

In field trials observed resistance was compared with resistance predicted from screening cultivars for this chemical factor. A rapid, simple technique requiring only small amounts of plant material was used, based on a 30s-extraction in chloroform and measurement of the intensity of the C_{32} peak by GC separation. Success was qualified by the need to consider also physical plant characters. However, the chemical technique used in conjunction with visual assessment of physical resistance factors could be usefully incorporated into resistance screening programmes for stemborer.

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EFFECTS OF HYDROXAMIC ACIDS ON THE RESISTANCE OF WHEAT TO THE APHID SITOBION AVENAE

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1. Introduction

Sitobion avenae F. is a sporadically damaging pest of wheat in temperate climates. Wheat extracts contain hydroxamic acids (Hx) which have ben shown to be important in resistance against insects in several Gramineae (Niemeyer & Perez, this symposium). The most abundant of these acids in wheat is 2, 4 - dihydroxy - 7- methoxy - 1, 4 - benzoxazin - 3 - one (DIMBOA). This compound has also been shown to be involved in the resistance of several wheat cultivars to a number of aphid species but resitance to **S. avenae** has not been investigated in the same context.

The main objective of this investigation was to assess a range of wheat cultivars, previously assessed for hydroxamic acid levels, in order to measure and rank them for antibiotic and antixenotic (non-preference) resitance to **S. avenae.**

2. Materials and methods

Seed samples of six wheat cultivars representing a range of known Hx levels were grown under permanent light at c.26°C with a 10°C range in a glasshouse at the University of Chile, and harvested at the two-leaf stage (G.S. 12; Zadoks). The Hx content of the tissue was estimated using a method based on the formation of a blue complex with ferric chloride solution (Bohidar et al., 1986).

For assessment of aphid performance at Southampton, seeds of the same cultivars were grown in conditions similar to the above. They were transferred after c.5 days to a culture room and kept at 20°C with a 2°C range, 60-70% r.h. and 16 h photoperiod.

Stock cultures of **S. avenae** were clonal, and were maintained on wheat (cv. Hobbit) in the culture room under the above conditions. Adult apterous viviparae of unknown age were placed individually on the test seedlings (30 of each cultivar) which were at the two-leaf stage; these were then covered with transparent plastic cylinders 30 cm high with a Terylene mesh top. These aphids were left for 24 h to reproduce and then removed together with all but two first-instar nymphs. The latter were left undisturbed until they moulted to the adult stage, but were checked daily during this period. One was then removed and the daily fecundity of the remaining singly-caged aphids (up to 30 on each cultivar) was recorded for 10 days. Progeny of these were removed daily, using a fine paint brush, during the same 2 h period per day.

The intrinsic rate of natural increase (r_m) was then calculated (Bohidar et al., 1986).

3. Results and discussion

Estimates of r_m were calculated at daily intervals. On all cultivars, nymph production during the first few days of reproduction contributed most to the value of r_m, a pattern similar, to those found for other aphid species. Values of r_m based on 10 days'recording were used in subsequent analysis.

There was a highly-significant negative relationship between the r_m value achieved on the cultivars and the concentration of hydroxamic acids in their tissues; the proportion of the variation in r_m values explained by acid levels was up to 96% depending on whether the variables were arithmetic or one, or both variables, were logarithmically transformed. The confidence limits for r_m were very low.

Of the components fo r_m, mortality did not begin on any cultivar until 16 days after birth; this was well after the highest daily fecundity had been reached (8-12 days after birth). This pattern agrees with that found by Frazer (1972) for two aphid species on **Vicia faba** L. cultivars. In addition to the correlation shown using logarithmically transformed variables, the age of the aphids at the time of the peak in age-specific fecundity was positively related to acid levels ($\log y=2.17 + 0.17 \log x$; $P<0.05$ $r=0.86$).

This preliminary investigation has revealed that hydroxamic acids appear to play a major role in seedling resistance to **Sitobion avenae.** Recent work has involved the study of antixenosis (non-preference) but no evidence has been found of any correlation between antixenosis and Hx content. We are now screening a wide genetic range of material for aphid resistance and hydroxamic acid content at seedling stage. Preliminary analysis by Niemeyer and Perez (this symposium) has shown extreme values of hydroxamic acid concentration are to be found in wild diploid species: highest in **Aegilops speltoides** and lowest in **A. squarrosa.** Modern polyploid wheats sharing a common genome had relatively uniform and low values. The ancient diploid species **Triticum monococcum** also had low levels. These results suggest that genomes B and G produce the wheats with high Hx concentrations. The genome D present in modern hexaploids may also have a suppresor affect. It is hoped that the wide genetic screen may reveal promising material suitable for incorporation into a plant breeding programme.

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CONCLUDING REMARKS

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Not quite ten years ago the conference on Insect Plant Relations held in Slough, England, concluded with an analysis of past developments and prospects for the future. At that time it was pointed out that unprecedented technical developments together with an increase in the number of workers in the vineyard had changed our philosophical approach to this field so that there was then a more respectful appreciation of the breadth, complexity, and nuances of the insect/plant relationship. The field is as broad as biology itself! This conference held at Pau, the sixth in the series, has not only confirmed that perception but has documented the wealth of experimental effort that has been expended in studying this newly appreciated breadth and complexity. Our intellectual forays into this field resemble the explorations of an amoeba which extends pseudopodia in many directions simultaneously, follows some, retracts others, seemingly does not know where it is going, but nevertheless makes progress.

Obviously one cannot summarize adequately in a few minutes the proceedings of a conference that has engaged our attention for four days. I shall endeavor, therefore, to comment in broader terms on those intellectual pseudopodia into which the corpus of our efforts seems to be flowing strongly. I shall not attempt to summarize the conclusions of 120 oral presentations and posters. Nor shall I be telling you anything that you do not already know; but perhaps by juxtaposing topics that were discussed at this conference and that traditionally might not appear to be related, I might be able to present some new perspectives.

Permit me first to set the stage. Since in the world of heterotrophs the "food" is the most often another living organism (exceptions being detritus feeders and necrophagous and coprophagous species) the relations between the consumer and the consumed are dynamically complicated, especially at the behavioral and ecological levels. Usually, however, when a prey is consumed, its only influences are effected posthumously; it affects the predator nutritionally in the broadest sense (i.e., provides calories, essential compounds, trace elements, etc.) and pharmacologically and toxicologically as well. The insect/plant association, on the other hand, is more realistically a parasite or symbiont host relationship. The plant (the host) in most cases continues to live, and it responds to attack physiologically so that parts not yet consumed may, at least locally, become different from the parts attacked initially. Thus the insect/plant

relationship differs from a prey/predator one in that the response to or prevention of attack by herbivores is chemical and physiological rather than behavioral (in the usual restricted sense of the word). The relationship is based, therefore, on interactions between the sensory system of a mobile attacker and the physical and chemical characteristics, together with physiological responses, of a stationary defender.

Since the initial encounter between an insect and a plant is a consequence of the initiative of the insect, a logical starting point for discussion would be foraging. For reasons which I hope will become apparent I shall defer consideration of foraging and commence with the actual encounter. From the insect's point of view this is a sensory phenomenon governed at the first stage by receptor physiology.

As this conference has shown, there is an interaction of several sensory modalities - vision, olfaction, and contact chemoreception. Tactile stimulation is still the neglected modality. The role of vision is assuming ever greater importance in our considerations, especially, though not exclusively, in respect to oviposition and foraging. The relevance of chemosensory physiology to insect/plant interactions continues, however, to attract most of our attention.

From an earlier concept of specialized chemoreceptors narrowly tuned to specific chemicals, of rather simple token stimuli that signaled the presence of appropriate plants, and of labeled neural lines that triggered specific behavior we had then years ago already begun to adopt the idea of receptors with broader action spectra operating not solely as labeled lines but in concert with other receptors to generate ensemble neural patterns (across-fiber patterns) which enable the insect to perceive the Gestalt of a plant. During the past four days we have witnessed both an expansion and a focusing of these concepts together with intensified efforts to relate receptor encoding and information transmission to reality in nature.

Formidable problems remain. Interpretation of electrophysiological signals is hampered by restrictions inherent in the methods of recording: the electrode is not always "seing" what the central nervous system "sees"; variability is a constant irritation; redundancy is poorly understood; multineuronal activity is a major source of confusion. Probably we shall not be able to relate sensory events to behavior with complete confidence until we learn to place electrodes in the central nervous system accurately and eavesdrop on the interneurons that collect and decode incoming messages.

Despite these restrictions we can, nevertheless, on the basis of newly acquired knowledge advance some provocative hypotheses. For example, by analogy with receptors of some non-herbivorous species we can say with reasonable assurance that even receptors as narrowly tuned as a sugar receptor may have multiple molecular sites each comprising a different proportion of the total population. Wa are thus presented with opportunities for proposing novel mechanisms to explain evolutionary

changes from one host preference to another and even from monophagy to polyphagy or vice-versa.

Consider, for example, a receptor that has membrane sites A, B, C,. etc. with which gustatory stimulants a, b, c etc. react specifically or preferentially. Imagine a simple case in which A is more numerous than other sites thus allowing a to be a more effective stimulus than b, c etc. The insect might be presumed, therefore, to prefer a to other compounds wherefore plants rich in a would be preferred over other plants even if a were part of a pattern. If a mutation were to occur eliminating site A, host preference, other things being equal, could shift to plants such in b, c etc. This scheme is, of course, an oversimplification; nevertheless, such a sequence of events has actually been demonstrated in the laboratory with **Drosophila** in which a mutation that deleted the site for the sugar threhalose rendered mutant flies insensitive to that sugar while retaining sensitivity to glucose and fructose. In passing it can be noted that this use of genetics in the service of understanding insect/plant relations represents one intellectual pseudopodium that appears to have aborted.

Another aspect of receptor physiology, which may be termed peripheral integration, has provided an opportunity to understand more fully the behavioral aspects of deterrency and repellency. At one time deterrents and repellents were believed to be unitary in their respective modes of action; that is, the prevailing view was that a single (or very few) magic chemical configurations effected behavioral rejection. Furthermore, rejection was believed to be the expression of sensitivity to a single, or at most two, modalities analogous to bitter and sour experienced by human beings. Both of these ideas have been challenged by electrophysiological and learning studies.

The unitary idea was a sterile hypothesis. In the absence of knowledge about receptors and after decades of fruitless screening of thousands of candidate deterrents and repellents the effort was largely abandoned. Clearly an entire galaxy of unrelated compounds can elicit behavioral rejection. Recent evidence of the diversity of feeding deterrents has been presented at this conference. Furthermore, it is now fully realized that what is a deterrent for one species is not necessarily a deterrent for another.

The concept of a unitary modality "rejection" has been called into question by new studies of deterrent receptors. It is conceivable that these receptors could be a specific as, for example, sugar receptors. It is also possible to imagine a multiplicity of sites to provide some breadth of sensitivity without sacrificing specificity. Where deterrent receptors have been found their relations to behavior have been clearly established either by electrophysiological or genetic studies. There have been, for example, mutant silkworm larvae in which deletion of deterrent receptors has abolished behavioral rejection.

The foregoing concepts envisioned a central integration by means of which feeding preferences are regulated by balance betwen feeding stimulants and deterrents. This idea is still valid; however, it must now be expanded to include peripheral integration. If I may select alkaloids as examples, there is a voluminous literature attesting to the fact that many of these compounds interfere with feeding but that the deterrent effect can be counteracted by increasing the concentration of accompanying feeding stimulants. This finding has far reaching significance because it indicates that the absolute concentration of a deterent in a plant is meaningful only in relation to the concentrations of sugars and other stimulants also present. At the same time electrophysiological studies have failed to detect any deterrent receptors in some species of insects even though those species were behaviorally deterred by the test compounds. The studies did reveal that many alkaloids inhibited sugar (and possibly salt and water) receptors, not only in those species lacking deterrent receptors but also in those species possessing them.

Early evidence suggested that alkaloids acted as competitive inhibitors. One problem with this suggestion was that of explaining how such widely diverse chemical structures could act competitively with such highly specific receptor sites as , for example, those for sucrose. The current view is that the reaction resembles narcosis or some general membrane phenomenon and that specific sites are not involved. Further investigation along these lines might provide a clearer understanding of the role of plant chemicals in insect/plant relationships. The idea of a peripheral integration that will accommodate a wide variety of chemical structures would allow for an evolutionary process by which behavioral deterrency could arise not only from the selection of specific deterrent receptors but by the development of acceptance receptors that were protected against inactivation by compounds acting as narcotics, competitors, and so forth.

Other analyses of receptor physiology, not discussed at this conference but bearing on problems that have been presented, have utilized information theory and investigations of difference thresholds (Weber fractions). They have confirmed behavioral observations that multiple receptor input and multimodal sensory activity (e.g., vision plus chemoreception and exteroceptive plus interoceptive input provide the insect with a wealth of information reflecting the complexity of its environment. By combining the principles of information theory with electrophysiological measurements it has been possible to assess the limits of the capacity of a receptor to transmit information. Yet, the whole organism clearly acts on the basis of a richer volume of information than any single receptor or category of receptors can transmit. Even though involvement of larger uniform populations of receptors and additional multimodal systems introduces more noise above which the signal must be heard, the ultimate result is more information. This expanded view enables us to appreciate more fully the importance of guarding against oversimplification of the insect/plant relationship. It cautions against

interpreting feeding, oviposition, and foraging behavior in terms of simple receptors and simple receptor systems alone. The insect marches to a more complexly orchestrated tune.

The source of much of the additional information governing the behavior of herbivores emanates from the internal milieu of the insect itself. The level of satiety is a case in point. Internal states alter not only the propensity for eating but also the breadth of the menu accepted. It is common knowledge that finickiness in feeding is inversely proportional to the level of deprivation. For example, higher levels of deterrent adulterants are accepted as deprivation increases. Where deterrent receptors are involved, central integration of positive and negative input is adjusted to a new set-point by the presence of absence of internal inhibition. Where peripheral integration occurs, its net input is balanced centrally against internal inhibition. Evidence has been presented at this conference that changes in peripheral sensory activity may also be influenced directly by internal states. In the final analysis what is important is the net volume of positive information that arrives at the command or motor neurons. An interesting question regarding mechanisms underlying finickiness and alteration of preferences remains unanswered, namely, why deprivation affects generalist feeders more than specialist feeders.

Thus far I have pictured the insect as a response system in which a behavioral event occurs strictly in conformity with conditions existing at the moment of execution. Indeed, ever since the classical studies of Jean Henri Fabre the insect has been cast in the role of an automaton ruled by blind instinct that only the stochastic wisdom of natural selection has guided. In modern electronic-age jargon the insect would be described an input-output machine. In later times we became aware that the central nervous system functioned in a more sophisticated capacity than that of a mere switching device which routed sensory information to motor command units. Even then, however, the system was conceived of as having only one time parameter-the present; that is, antecedent neural and behavioral events had no effect beyond their time. But by then elegant experiments with Hymenoptera revealed that those particular little automatons are capable of quite respectable learning. Diptera and herbivorous insects in general continued to be treated as the dunces of the class. All that has now changed because it is we who have learned.

The behavior of many phytophagous insects towards host plants is clearly influenced by prior experience. Revelation first of induction and then of aversion-learning has been followed, as this conference documents, by exposition of other kinds of experiential behavior modification, by habituation, and by genuine classical conditioning. Classical conditioning seems at the moment to be restricted to, or at least most highly developed in, flying forms, especially in relation to pollination and oviposition. Its apparent absence in grazing forms may represent only our lack of success thus far in uncovering it.

The potential for learning introduces another facet of insect/plant relations and raises provocative ecological and evolutionary questions. one of the most interesting, to which I propose no answer, is: what is the selective advantage of learning in this context?

All the interactions discussed in the foregoing paragraphs are those concerned with behaviors triggering ingestion and oviposition. Events preceding and setting the stage for these consummatory actions are primarily aspects of locomotory behavior and may be addressed at three dimensional levels: habitati, patch, and plant.

At the level of habitat the interaction is primarily between the insect and the general environment (of which the plant is a contributing unit but is not a direct interacting participant). Once the plant has come within sensory range, whether this be visual of olfactory, there is a direct insect/plant interaction. The signal indicating the presence of a plant, or a specific plant, now affects locomotory behavior. At this juncture the distribution, density, and community composition of plants may become relevant stimuli. Finally, on the plant itself the insect continues its exploration because, for reasons little understood, no all parts of a plant, or indeed even of a single leaf, are equally acceptable. In short, foraging involves all these locomotory excursions, and the plant itself may be directly involved only in the last two.

Studies of foraging previously have concentrated more on theoretical aspects than real. A great deal of mathematical modeling has been done. Emphasis has often been placed on energetic aspects especially with respect to energy gained for energy expended. This has led to theories involving expectations, rate of reward, etc., many based on the assumption of memory and/or foreknowledge, characteristics of prey/predator relationships, behavioral abilities of vertebrates with more complex nervous systems, and the mobility of flying species with comparatively long range sensory scanning abilities. Only more recently have intensive behavioral studies been undertaken. Still, few studies have been conducted with the more sedentary herbivores. Furthermore, scant attention has been paid to the limitations imposed by sensory capabilities. And the emphasis continues to be placed upon populations rather than individuals.

Once we escaped from the concepts of simple receptors, token stimuli, rigidly programmed input/output insects, energy input/output ratios, and static non-responding hosts as the predominant determinants in the insect/plant relationship we began to appreciate more fully the exquisite interation between plants, the labile chemical repertoire of which becomes ever more complex as our analytical prowess increases, and insects, whose physiological and ecological needs require more than colories. The insect does not live by plant nutrients alone as studies of the allocation of some ingested compounds to the synthesis of pheromones and defense compounds show.

Knowledge of all these newly revealed capacities of plants and insects forces us to reexamine our ideas of ecological relationships and evolutionary hypotheses. We cannot fully comprehend what populations are doing without understanding individuals nor what individuals are doing without some understanding of relevant features of their physiology and behavior. To that end periodic conferences are necessary. This conference at Pau has opened many closed doors, put our thinking to the test, and raised exciting challenges.

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