INSECTS-PLANTS '89

Symposia Biologica Hungarica

39

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Akadémiai Kiadó, Budapest

INSECTS-PLANTS '89

Edited by
Á. SZENTESI, T. JERMY

(Symposia Biologica Hungarica 39)

At the seventh international symposium of this series 135 participants from 22 countries discussed problems of host finding, host acceptance, nutrition and sensory physiology, host-related behaviour, ecology, evolution, etc. of phytophagous insects. The answers to the above questions were also multidisciplinary: given by behavioural geneticists, evolutionary ecologists, electrophysiologists, and not least by students of practice dealing with biological control and plant resistance to insects. While a treasure of extremely valuable new knowledge is presented in this volume, it is also clearly demonstrated what further questions are to be answered for a full understanding of the intimate connection between an insect and a plant.

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BUDAPEST

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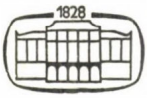

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PREFACE

The breathtaking diversity of insect world has always attracted and trapped a considerable size of scientific community to the domain of insect/plant relationships. Any attempts trying to enlighten facets of this intricate connection is subject to compromises among different disciplines. Indeed, what we today know as insect/plant relationship is a multidisciplinary approach by behavioural, physiological, ecological, phytochemical and evolutionary sciences to understand how and why insects select plant species for feeding, oviposition, hiding, advertising themselves, etc. to mention only some of the most apparent functions. The insect/plant symposia have, from the beginning, reflected this manner and the present one was no exception either.

The seventh in the series of International Symposia of Insect-Plant Relationships followed almost exactly after 15 years the third one that was organized also in Hungary (Tihany, June 9-14, 1974). A short comparison of the two meetings reflects the trends of development in this field of science.

Already the number of participants is interesting to compare. While in 1974 only 66 participants were present from abroad, in 1989 the number of foreign attendants reached 135, however, it would have been considerably higher if the organizers were not obliged to restrict the number of participants because of limitations in time and space and in order to provide ample opportunity for discussions. Judging from the skyrocketing number of publications issued yearly, one can suspect that the size of the scientific community in this field may exceed with orders of magnitude the number having attended the symposium.

It was unfortunate that the participants of the 1989 symposium had to miss the presence of several outstanding scientists of the older generation who attended almost all previous meetings. On the other hand, this meeting was characterized by the presence of many young scientists and students representing the future of the symposia and warranting the continuation of interest in this domain.

The various aspects of insect-plant relationships received different emphasis in the two symposia. The recent symposium was characterised by a considerably greater proportion of presentations in ecology and evolution. Futhermore, the problems of the third trophic level as well as behavioural genetics, behavioural plasticity (lear-

ning) and dietary self selection were new ideas that were greatly neglected or not even mentioned fifteen years ago.

The reader will find three types of contributions in this volume, besides the opening and closing presentations. Introductory lectures of daytime sessions or evening workshops reviewed the most important achievements of a given field. The so-called "ordinary" lectures (the name refers only to the length of the oral communication) and the posters represent the bulk of presentations. In the course of the preparation of the symposium the Organizing Committee - together with the International Advisory Board - had to face the embarassing task of asking several would-be participants who wanted to give a lecture, to present a poster instead. Otherwise parallel sessions should have been held in the course of the whole symposium, which would have strongly limited discussions.

The organization of the present symposium was greatly helped by the members of an International Advisory Board. Outstanding support was received from Prof. Louis Schoonhoven. The organizers are grateful to all colleagues for their assistance. In order to improve the preparation of the future symposia, the Dutch group of participants proposed to organize a permanent committee for the International Symposia on Insect-Plant Relationships, and this proposal was accepted by the participants. Its members will be recruited from the countries where research is carried out in this field.

Budapest, July 1989

The Editors

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OPENING ADDRESS

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INSECTS AND HOST PLANTS: 100 YEARS OF "BOTANICAL INSTINCT"

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ABSTRACT

Scientists, in their efforts to unravel the causal elements constituting the "botanical sense" of herbivorous insects, have made considerable progress since this instinct was described by J. P. Fabre in eloquent prose one century ago. The interplay between the almost incomprehensible chemical diversity of the plant Kingdom and the delicately tuned chemosensory systems in insects seems to be a key element of the botanical instinct. We have begun to appreciate, however, that rather than being fixed entities, insect behaviour and plant chemistry are both dynamic systems involving behavioural plasticity and genotypic adaptations on the insects's side, and complex physiological plasticity on the plant's side. The interaction between the two systems can be investigated from different viewpoints, such as a mechanistic analysis, a genetic approach, and an evolutionary ecological (cost-benefit) model. Our insights are developing more or less rapidly, depending on the approach chosen. The ultimate goal is a synthesis of knowledge obtained via different routes, leading to a greater understanding of insect-plant relationships, and an explanation of the intricate principles underlying an insect's botanical sense.

Key words: "botanical instinct", "botanical sense", herbivorous insects, insect-plant relations, phytochemistry, phagostimulant, deterrent, chemoreceptor, brain, central nervous system, experience, induction of food preference, physiological feedback.

INTRODUCTION

No animal life exists in the absence of green plants, since they serve as the primary source of energy-rich compounds for all heterotrophic organisms. On the other hand, the present striking diversity of the plant kingdom is the result of a long-lasting co-habitation with animals. Among them, insects with their numerous forms and habits are the prime effectors of plant evolution. Therefore, the insect-plant "interface" may be the major zone of interaction responsible for generating terrestrial organic diversity.

A multitude of ecological studies has revealed that insects and plants do not simply live together, but rather that they interact with each other, they suffer from each other, they adapt to each other, because they need each other and they both are as they are because of their coexistence. The natural world would change its appearance rapidly and drastically if all insects were to be removed.

The topic of insect-plant relationships has captured the attention of naturalists and agriculturists since antiquity, though a comprehension of the presence of some fundamental interactions between insects and plants may be dated from 1793, when the German botanist C.K. Sprengel discovered that insects have a starring role in the fertilization process of many flowering plants.

A basic concept resulting from the study of insect-plant relations is the notion that herbivorous insects are specialist feeders, which implies that even the relatively few so-called polyphagous species have a limited diet. Curious entomologists wanting to explain how an insect recognized its specific food plants, were for a long time content with the view that it finds its way "led by an instinct far more unerring than the practised eye of the botanist" which it possesses because "she is taught of God" (Kirby and Spence, 1863). Almost exactly 100 years ago J.H. Fabre (1886) in his eloquent prose elaborated on this botanical instinct. Fabre's writings have challenged many scientists to unravel the delicate choosiness of herbivorous insects. Although as a result several significant contributions to our present understanding of the causal elements constituting their botanical sense have been made, progress has been undeniably slow. The topic of insect-plant relationships crosses the traditional boundary between zoology and botany and probably therefore did not gain momentum until fairly recently. Insect-plant relationships are now receiving rapidly increasing interest as is evidenced by the considerable number of books being published in this area. Half of all books on this subject were published during the last 5 years. The appearance of so many publications indicates the need to synthesize our knowledge and to order the multitude of known facts into abstractions and principles underlying the relationships between arthropods and angiosperms, the two largest phyla in the living world.

PHYTOCHEMISTRY: THE BASIS OF BOTANICAL INSTINCT

The term "botanical instinct" has been used with two slightly different connotations. In its more restricted meaning it implies that an insect in some way or other recognizes its host plant amidst other plant species. In a broader sense it is used to indicate that an insect, feeding on more than one plant species, has a sort of "sixth sense" which enables it to recognize the taxonomic affinities of plants, and thus restricts its attacks to related plants only.

Due to the work of phytochemists we now recognize that the basis of the esoteric botanical sense is to be found in the chemical relatednesss of plants belonging to a certain taxon. The two meanings of the term botanical instinct are compatible with each other when we realize that taxonomic relationship is in many cases synonymous with chemical relationship and that insects do not search for plants that are classified within a certain taxon, but for all those plants which fit into a certain chemical profile. This profile may be narrow and confined to one plant species, or it may be somewhat broader and cover a plant genus or even family.

The famous and often cited experiments by Verschaffelt (1910) on the role of several host-specific chemicals as feeding stimulants for some specialized insects evidenced the presence and importance of a well tuned sense of taste. The classical observations by Dethier (1941) on the attractiveness of specific mixtures of essential oils to swallowtail butterfly larvae revealed the existence of an olfactory sense, capable of acute discrimination.

It is just 100 years ago when some botanists launched the idea that secondary plant compounds have a protective function: "many of the chemicals may serve the plant as means of defence against animals, and when we camphorize our furniture and poison our flower-beds, we are only imitating and reinventing what the plants practised before the existence of man" (Abbott, 1887). Our since then gradually growing knowledge of the chemical diversity among plants especially with regard to the wealth of secondary compounds they harbour (e.g. Hegnauer, 1962-1986; Smith, 1976), led us to confer on them an increasingly important role in insect-plant relationships, as exemplified by G.S. Fraenkel's well known 1959 and 1969 papers. At present we consider a plant's chemical constitution as the primary factor determining insect-plant relationships, as illustrated by the dictum: "Many factors influence the evolution of herbivore diets, but plant chemistry is central" (Schultz, 1988).

It is curious how, despite an intensive search, relatively few specific feeding stimulants have been discovered. Jermy (1958) was among the first to voice the importance of avoiding feeding deterrents in food recognition behaviour, and developed the opinion that "host plant specificity in phytophagous insects is determined mainly by the botanical distribution of plant substances inhibiting feeding..." (Jermy, 1983). For a long time I found this idea hard to accept, since to me it seemed a much more efficient and reliable strategy for an insect to recognize its exclusive food by the presence of a specific positive signal, i.e. a phagostimulant. But there is growing support for Jermy's statement (Bernays and Chapman, 1987). This raises the question as to whether plant selection behaviour based on avoiding negative stimuli is a better tactic than hunting for positive stimuli. Or is the observed imbalance between the insects primarily searching for phagostimulants and those evading deterrents merely a result of our misjudgement due to experimental limitations?

THE ROLE OF CHEMORECEPTION

The mystery of the botanical instinct has now been reduced to the problem of how the chemical profile of a plant is perceived by the insect sufficiently accurately that it can choose unerringly certain plants and consistently ignore all others. The existence of generalized feeding deterrent receptors (Ishikawa, 1966; Ma, 1969) as well as some highly specific phagostimulant cells (Schoonhoven, 1967a; Rees, 1969) was discovered in some insect species during the sixties. Almost immediately sensory physiologists realized that the sensory information an insect receives upon contact with the plant interior must be much more complex than a simple message conveyed by one or two specific cells, or "labelled lines". There is increasing evidence that

natural stimuli, such as plant contents, act as a whole. For instance, the capacity of sinigrin to stimulate feeding in the garlic mustard inhabiting weevil *Ceutorhynchus constrictus* manifests itself only in the presence of other leaf constituents (Nielsen et al., 1989). Obviously complex stimuli evoke intricate neural patterns in the insect's chemoreceptors (Dethier and Kuch, 1971; Städler, 1986; Schoonhoven, 1987).

Since we may assume that the brain conducts behavioural output on the basis of sensory input, diligent attempts have been made to decipher the sensory code and to fathom its message. Simple quantitative relationships appeared to exist between neural activity in some chemoreceptors and feeding intensity. Moreover, the brain seems to summate the incoming impulses from different receptors algebraically, although impulses in different lines are given different weighting (Schoonhoven and Blom, 1988). This seemingly simple situation, however, is complicated by several factors. To mention a few: receptors often have overlapping reaction spectra; mixtures of natural chemicals may cause receptor modulations which are unpredictable on the basis of observations with single chemicals (Schoonhoven, 1987; de Jong and Visser, 1988a); in some way or other the dimension of time plays a role, as evidenced by different stimuli causing different temporal patterns (Dethier and Crnjar, 1982). In the Colorado potato beetle de Jong and Visser (1988b) obtained evidence that olfactory information is processed in the deutocerebrum in two different ways. One channel analyses information on stimulus quantity, the other one evaluates stimulus quality.

Progress in unravelling the sensory code has been, due to these complications, disappointingly slow. Even in "simple" systems, as found in caterpillars with only a few tens of taste cells, the sensory message proves to be too complex to be solved easily. Computer programmes as developed recently (Frazier and Hanson, 1986; Smith et al., 1989) may add a new tool to our attempts to discover the intricacies of the sensory code. When we are able to crack this code we will probably understand the quintessence of the botanical instinct.

THE ROLE OF EXPERIENCE

There is a complication, however, in that neither behaviour nor relevant physiological parameters are constant entities. Not only do interindividual variations occur, which physiologists usually average and then tend to neglect, but also individuals may show inconstant physiological responses and variable behaviour. For instance, host preferences have been found to be modifiable, and we must therefore conclude that the botanical instinct shows some plasticity. Conditioning to certain food plants was already observed by Réaumur, 250 years ago: "It is worthy of remark, however, that when some of these [i.e. polyphagous herbivores] have fed for a time on one plant they will die rather than eat another, which would have been perfectly acceptable to them if accustomed to it from the first." (Kirby and Spence, 1863, p.219). The vagueness of the description of this phenomenon was reduced considerably in the hands of Jermy and co-workers (1968), when in a classical study they succeeded

in quantifying food preference induction. Thus the feeding preferences hitherto presumed to be inborn and engraved in the insect's central nervous system, appeared to be modifiable, even to the extent that under certain conditions non-host plants become acceptable. Tobacco hornworm larvae, for example, accept after being grown on an artificial diet, cabbage and dandelion, plants which do not belong to their normal host plant range (Schoonhoven, 1967b). Likewise insects normally repelled by the smell of citral, a natural compound present in citrus fruits, may be conditioned to this substance and are then even attracted to it (Saxena and Schoonhoven, 1978). Several more observations made mostly during the last two decades, suggest that experience may play a considerable role in subtle details of host plant finding (Saxena and Schoonhoven, 1978), acceptance (Blaney et al., 1985), and preference (de Boer and Hanson, 1984). Jermy (1987) in a lucid paper on this subject recently came to the conclusion that "induced feeding and oviposition preferences are nothing other than a further restriction in the overall extreme finickiness fo phytophagous insect species at the population and/or individual levels". The fact that the induced preference is the more pronounced when the insect is tested against taxonomically more dissimilar plants may be again explained by the concept that taxonomically related plants often have similar natural product plant chemistry, whereas unrelated plant taxa usually are quite different in this respect (de Boer and Hanson, 1984).

Concomitantly with changes in preference behaviour, the sensory message conveyed to the brain appeared to be changed as a result of feeding history (Schoonhoven et al., 1987). Apparently the primary informants of the brain, the chemoreceptors, also possess a certain degree of adjustability. These findings, showing that sensory input as well as behavioural output can be modified depending on previous experience, necessarily alter our view on the botanical instinct. Instead of a fixed entity it has to be regarded as a command system which to a certain extent can be reprogrammed. Thus the botanical instinct comprises a species-specific receptor system, tuned to discriminate the chemical profile of its host or hosts from non-hosts, complemented by an integration centre in the central nervous system harmonised to read the incoming sensory message, as well as a well-defined degree of programmability of the whole system.

VARIATIONS IN PLANT CHEMISTRY

Entomologists have often, for the sake of simplicity, considered plants as chemically fairly rigid and constant systems, each species characterized by its nutritional value and its specific biochemical fingerprint. As so often in biology this fixed image appeared to be too simple and even deceptive. Naturally, plants, even when belonging to the same species, may show large genotypic and phenotypic differences. These variations are undoubtedly of paramount importance to the insects inhabiting them (as was recognized a long time ago by plant breeders selecting resistant varieties of our main crop plants). An extra dimension to this chemical diversity was discovered when Ryan (1983) working on perennial plants, and Haukioja and Niemelä (1977)

on trees, showed that insect infestation may trigger the activation of a chemical defence system. Palatability and nutritional value can be severely reduced even at relatively low insect densities, resulting in retarded insect growth and considerably increased mortality (Wratten et al., 1988). The plant thus appears to be an active participant in its own defence, rather than a static and passive victim of insect attacks. Obviously the fact that both plant chemistry and insect behaviour show appreciable plasticity will seriously complicate the analysis of their interactions.

Once we recognize that in all interactions there exists (besides the inherited component) considerable phenotypic plasticity, the need to define and delineate the genetic component becomes still more compelling. A genetic analysis may help to explain not only interindividual variations in host acceptance within one population, but also the differences in host preferences for feeding and oviposition observed in different populations of the same insect. Such differences may be related to geographical distance, as for example in the small ermine moth, *Yponomeuta padellus*, which when collected in Finland prefers to oviposit on mountain ash (*Sorbus aucuparia*), whereas moth originating from the Netherlands prefer hawthorn (*Crataegus monogyna*) for oviposition (van de Water, 1982). Interestingly, considerable differences in oviposition preferences have also been found between different populations of a butterfly species within the same region and living in apparently identical habitats (Singer, 1984). Clearly, there is a great need for more genetical studies as has been emphasized before by e.g. Dethier (1978) and Futuyma and Peterson (1985). The genetical approach is particularly relevant for understanding host shifts, which imply a dramatic change of an insect's botanical instinct. In particular studies in which a genetical analysis is combined with sensory physiology, as well as nutritional aspects and insect feeding behaviour could prove to be very fruitful in grasping the essence of host shifts, a major event in the evolution of insect plant-relationships (Thompson, 1988).

Natural selection may influence the botanical instinct of a species resulting in behavioural differences between groups of individuals within a population. For example, different strains of an insect species may possess genetically fixed differences in their olfactory system (Visser, 1983) or in the sensitivity of their taste cells (Wieczorek, 1976). Behavioural differences may also be located in the central nervous system, as is the case in the polyphagous strain of the common silkworm, which shows marked changes in food acceptance, despite the fact that its chemoreceptors produce the same sensory message as the normal strain, accepting only mulberry (Ishikawa et al., 1963).

FOOD PREFERENCE IS INFLUENCED BY PHYSIOLOGICAL FEEDBACK

Recently a remarkable new feature of insect feeding behaviour was discovered, which hitherto was only known from vertebrates. When caterpillars are fed a suboptimal diet, which, for example, is short of proteins or carbohydrates, they subsequently select in a choice situation a food which compensates for the deficiency

(Waldbauer and Friedman, 1988). In the migratory locust such compensation behaviour is shown after only a single meal of the incomplete food (Simpson et al., 1989), indicating the presence of a very rapid and sensitive feedback system, which translates nutritional inadequancy into a signal modifiying the CNS food selection centre. In this context an interesting experiment by Dethier (1980, 1988) may be mentioned. When a polyphagous caterpillar was fed for some time on an initially acceptable, but toxic plant species, the insect fell ill. After recovery from intoxication its threshold for accepting this plant species was changed, and the toxic plant was partially or completely rejected. Apparently the temporary physiological malaise was associated with the type of food ingested (Bernays and Lee, 1988). These experiments shows that general intoxication or lack of specific nutrients is associated with certain food type and that rapid feedback mechanisms occur, which affect food choice, i.e. act on the botanical instinct. The fact that little or nothing is known about the nature of the signals involved in this feedback system (but see Abisgold and Simpson, 1987), nor about the internal receptors transmitting the information to the brain, indicates that we still have only a fragmentary knowledge of the physiology of insect-plant interactions.

When Fabre, a century ago, introduced the term "botanical instinct", he merely used it as a shorthand term for a complex and unfathomable behaviour. The concept of instinct was at that time predominantly used as a convenient vehicle for describing nonrational elements of behaviour. Instinctive activities were seen to be inborn, reflex-like and driven from within. The concept of instinct, however, has since undergone many changes and has diminished in importance. Modern ethology has separated out the innate, the reflex and the motivational aspects of apparently instinctive behaviour, and these are now regarded as separate issues.

Our current view of an insect's botanical instinct incorporates present concepts of the behavioural sciences, such as the postulate of motivation and the role of experience in ontogeny.

BOTANICAL INSTINCT: A THREE-TIER SYSTEM

As in Fabre's days an insect's botanical instinct is seen to manifest itself by its predilection for one or a few, often related plant species, in combination with the rejection of non-hosts. We now recognize that this behaviour involves three major elements. (1) A peripheral chemoreceptor system, sensitive to multiple chemical stimuli, composed of phagostimulants and deterrents. The response spectra of the chemoreceptors are characteristic of each insect species. (2) A central nervous system tuned in such a way as to recognize sensory patterns. Certain patterns are identified as acceptable, i.e. they release feeding or oviposition behaviour (which may be energized by a motivation centre), others promote rejection. The decision centre for feeding may be located in the suboesophageal ganglion. Possibly it consits of only a single decision-making interneuron, as is the case in some other discrete and identifiable acts in insects, as found in flying crickets, in which a single sensory interneuron

elicits avoidance steering behaviour in response to ultrasounds (Nolen and Hoy, 1984). (3) An internal chemosensitive system which measures the quality and quantity of absorbed food constituents and which warns the central nervous system if food composition differs too much from physiological requirements, resulting in a change of food selection. The former two elements in this system, the receptors and the CNS, can be modified in response to previous experiences. Possibly the same applies to the internal feedback mechanism.

Needless to say, the botanical instinct is in many ways intertwined with an insect's physiological capabilities. For example food generalists have higher levels of detoxifying enzymes than food specialists (Krieger et al., 1971) and compounds such as glucosinolates, which stimulate food intake in insects specialized on crucifers, are toxic to insects which normally feed on other plants (Erickson and Feeny, 1974). Several other physiological variables, like age, dietary history, sex and temperature may influence food choice (Blaney and Simmonds, 1987; Schoonhoven et al., 1987).

The botanical instinct as defined in the light of modern knowledge may seem to have lost some of its original mysterious connotation. We appreciate its three interacting elements of receptors, central nervous system, and nutritional feedback, the whole perpetually interacting with numerous ecological constraints. In spite of all our investigations, the botanical instinct of insects remains an enigma, and the knowledge gained in 100 years has only served to alter the questions being asked. We now wonder what at the molecular level determines the almost infinite variation in chemoreceptor specificity. We wonder how experience modifies the neuronal networks responsible for acceptance or rejection of certain plants, and we hardly know how to analyse the problem of why some insects show an extreme finickiness, whereas others tolerate a range of hosts. Unquestionably the knowledge gained since Fabre's days has widened our horizons, and gratified our curiosity, but the problem of the botanical instinct remains in essence unsolved.

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REFERENCES

Abbott, H. C. de S. (1887): Comparative chemistry of higher and lower plants. *Am. Nat. 21*, 800-810.

Abisgold, J. D. and S. J. Simpson (1987): The physiology of compensation by locusts for changes in dietary protein. *J. Exp. Biol. 129*, 329-346.

Bernays, E. A. and R. F. Chapman (1987): The evolution of deterrent responses in plant-feeding insects. In: R. F. Chapman, E. A. Bernays and J. G. Stoffolano

(eds), *Perspectives in Chemoreception and Behavior*. Springer Verlag, New York. 159-173.

Bernays,E. A. and J. C. Lee (1988): Food aversion learning in the polyphagous grasshopper, *Schistocerca americana. Physiol. Entomol. 13*, 131-137.

Blaney, W. M. and M. S. J. Simmonds (1987): Experience: a modifier of neural and behavioural sensitivity. In: V. Labeyrie. G. Fabres and D. Lachaise (eds), *Insects – Plants. Proc. 6th Int. Symp. Insect-Plant Relationships.* W. Junk Publishers, Dordrecht. 237-241.

Blaney, W. M., C. Winstanley and M. S. J. Simmonds (1985): Food selection by locusts: An analysis of rejection behaviour. *Entomol. exp. appl. 38*, 35-40.

de Boer, G. and F. E. Hanson (1984): Foodplant selection and induction of feeding preference among host and non-host plants in larvae of the tobacco hornworm, *Manduca sexta. Entomol. exp. appl. 35*, 177-193.

de Jong, R. and J. H. Visser (1988a): Specificity-related suppression of responses to binary mixtures in olfactory receptors of the Colorado potato beetle. *Brain Res. 447*, 18-24.

de Jong, R. and J. H. Visser (1988b): Integration of olfactory information in the Colorado potato beetle brain. *Brain Res. 447*, 10-17.

Dethier, V. G. (1941): The function of the antennal receptors in lepidopterous larvae. *Biol. Bull. Woods Hole, Mass. 80*, 403-414.

Dethier, V. G. (1978): Studies on insect/plant relationships – past and future. *Entomol. exp. appl. 24*, 759-766.

Dethier, V. G. (1980): Food-aversion learning in two polyphagous caterpillars, *Diacrisia virginica* and *Estigmene congrua. Physiol. Entomol. 5*, 321-325.

Dethier, V. G. (1988): Induction and aversion-learning in polyphagous arctiid larvae (Lepidoptera) in an ecological setting. *Can. Entomol. 120*, 125-131.

Dethier, V. G. and R. M. Crnjar (1982): Candidate codes in the gustatory system of caterpillars. *J. Gen. Physiol. 79*, 543-569.

Dethier, V. G. and J. H. Kuch (1971): Electrophysiological studies of gustation in lepidopterous larvae. I. Comparative sensitivity to sugars, amino acids, and glycosides. *Z. vergl. Physiol. 72*, 343-363.

Erickson, P. R. and P. Feeny (1974): Sinigrin: a chemical barrier to the black swallowtail butterfly, *Papilio polyxenes. Ecology 55*, 103-111.

Fabre, J. H. (1886): *Souvenirs Entomologiques.* Vol. 3. Delagrave, Paris.

Fraenkel, G. (1959): The raison d'être of secondary plant substances. *Science 129*, 1466-1470.

Fraenkel, G. (1969): Evaluation of our thoughts on secondary plant substances. *Entomol. exp. appl. 12*, 473-486.

Frazier, J. L. and F. E. Hanson (1986): Electrophysiological recording and analysis of insect chemosensory responses. In: J. R. Miller and T. A. Miller (eds), *Insect-Plant Interactions*. Springer Verlag, New York. 285-330.

Futuyma, D. J. and S. C. Peterson (1985): Genetic variation in the use of resources by insects. *Ann. Rev. Entomol. 30*. 217-238.

Haukioja, E. and P. Niemelä (1977): Retarded growth of a geometrid larva after mechanical damage to leaves of its host tree. *Ann. Zool. Fenn. 14,* 48-52.

Hegnauer, R. (1962-1986): *Chemotaxonomie der Pflanzen.* Vol. 1-7. Birkhäuser Verlag, Basel.

Ishikawa, S. (1966): Electrical response and function of a bitter receptor associated with the maxillary sensilla of the silkworm, *Bombyx mori* L. *J. Cell. Physiol. 67,* 1-11.

Ishikawa, S., Y. Tazima and Y. Hirao (1963): Responses of the chemoreceptors of maxillary sensory hairs in a "Non-preference" mutant of the silkworm. *J. Sericult. Sci. Japan 32,* 125-129.

Jermy, T. (1958): Untersuchungen über Auffinden und Wahl der Nahrung beim Kartoffelkäfer (*Leptinotarsa decemlineata* Say). *Entomol. exp. appl. 1,* 197-208.

Jermy, T. (1983): Multiplicity of insects antifeedants in plants. In: D. L. Whitehead and W. S. Bowers (eds), *Natural Products for Innovative Pest Management.* Pergamon Press, Oxford. 223-236.

Jermy, T. (1987): The role of experience in the host selection of phytophagous insects. In: R.F. Chapman, E.A. Bernays and J.G. Stoffolano (eds), *Perspectives in Chemoreception and Behavior.* Springer Verlag, New York. 143-157.

Jermy, T., F. E. Hanson and V. G. Dethier (1968): Induction of specific food preference in lepidopterous larvae. *Entomol. exp. appl. 11,* 211-230.

Kirby, W. and W. Spence (1863): *An Introduction to Entomology.* Longman, London.

Krieger, R. I., P. P. Feeny and C. F. Wilkinson (1971): Detoxication enzymes in the guts of caterpillars: An evolutionary answer to plant defenses? *Science 172,* 579-581.

Ma, Wei-Chun (1969): Some properties of gustation in the larva of *Pieris brassicae. Entomol. exp. appl. 12,* 584-590.

Nielsen, J. K., A. M. Kirkeby-Thomsen and M. K. Petersen (1989): Hostplant recognition in monophagous weevils: specificity in feeding responses of *Ceutorhynchus constrictus* and the variable effect of sinigrin. *Entomol. exp. appl. 53,* 157-166.

Nolen, T. G. and R. R. Hoy (1984): Initiation of behavior by single neurons: the role of behavioral context. *Science 226,* 992-994.

Rees, C. J. C. (1969): Chemoreceptor specificity associated with choice of feeding site by the beetle *Chrysolina brunsvicensis* on its foodplant, *Hypericum hirsutum. Entomol. exp. appl. 12,* 565-583.

Ryan, C. A. (1983): Insect-induced chemical signals regulating natural plant protection responses. In: R. F. Denno and M. S. McClure (eds), *Variable Plants and Herbivores in Natural and Managed Systems.* Academic Press, New York. 43-60.

Saxena, K. and L. M. Schoonhoven (1978): Induction of orientational and feeding preferences in *Manduca sexta* larvae for an artificial diet containing citral. *Entomol. exp. appl. 23,* 72-78.

Simpson, S. J., M. S. J. Simmonds, W. M. Blaney and J. P. Jones (1989): Compensatory dietary selection occurs in larval *Locusta migratoria* but not *Spodoptera littoralis* after a single deficient meal during *ad libitum* feeding. *Physiol. Entomol.* (in press)

Schoonhoven, L. M. (1967a): Chemoreception of mustard oil glucosides in larvae of *Pieris brassicae. Proc. Kon. Ned. Akad. Wet. C 70*, 556-568.

Schoonhoven, L. M. (1967b): Loss of hostplant specificity by *Manduca sexta* after rearing on an artificial diet. *Entomol. exp. appl. 10*, 270-272.

Schoonhoven, L. M. (1987): What makes a caterpillar eat? The sensory code underlying feeding behavior. In: R. F. Chapman, E. A. Bernays and J. G. Stoffolano (eds), *Perspectives in Chemoreception and Behavior*. Springer Verlag, New York. 69-97.

Schoonhoven, L. M., W. M. Blaney and M. S. J. Simmonds (1987): Inconstancies of chemoreceptor sensivities. In: V. Labeyrie, G. Fabres and D. Lachaise (eds), *Insects – Plants. Proc. 6th Int. Symp. Insect-Plant Relationships*. W. Junk Publishers, Dordrecht. 141-145.

Schoonhoven, L. M. and F. Blom (1988): Chemoreception and feeding behaviour in a caterpillar: towards a model of brain functioning in insects. *Entomol. exp. appl. 49*, 123-129.

Schultz, J. C. (1988): Many factors influence the evolution of herbivore diets, but plant chemistry is central. *Ecology 69*, 896-897.

Singer, M. C. (1984): Butterfly-hostplant relationships: host quality, adult choice and larval success. In: R. I. Vane-Wright and P. R. Ackery (eds), *The Biology of Butterflies*. Academic Press, London. 81-88.

Smith, J. J. B., B. K. Mitchell, B. M. Rolseth, A. T. Whitehead and P. J. Albert (1989): SAPID tools: Microcomputer programs for analysis of multi-unit nerve recordings. *Chem. Senses* (in press)

Smith, P. M. (1976): *The Chemotaxonomy of Plants.* E. Arnold, London.

Sprengel, C. K. (1793): *Das entdeckte Geheimniss der Natur im Bau und in der Befruchtung der Blumen.* Vieweg, Berlin.

Städler, E. (1986): Oviposition and feeding stimuli in leaf surface waxes. In: B. Juniper and R. Southwood (eds), *Insects and the Plant Surface*. E. Arnold, London. 105-121.

Thompson, J. N. (1988): Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomol. exp. appl. 47*, 3-14.

van de Water, T. P. M. (1982): Allopatric speciation in the small ermine moth *Yponomeuta padellus*. In: J. H. Visser and A. K. Minks (eds), *Proc. 5th Intern. Symp. Insect-Plant Relationships.* Pudoc, Wageningen. 405-406.

Verschaffelt, E. (1910): The cause determining the selection of food in some herbivorous insects. *Proc. K. Ned. Akad. Wet. 13*, 536-542.

Visser, J. H.(1983): Differential sensory perceptions of plant compounds by insects. In: P. A. Hedin (ed), *Plant Resistance to Insects*. ACS Symposium 208, American Chemical Society, Washington, 215-230.

Waldbauer, G. P. and S. Friedman (1988): Dietary self-selection by insects. In: F. Sehnal, A. Zabza and D. L. Denlinger (eds), *Endocrinological Frontiers in Physiological Insect Ecology*. Wroclaw Technical University Press, Wroclaw. 403-422.

Wieczorek, H. (1976): The glycoside receptor of the larva of *Mamestra brassicae* L. (Lepidoptera, Noctuidae). *J. Comp. Physiol. 106*, 153-176.
Wratten, S. D., P. J. Edwards and L. Winder (1988): Insect herbivory in relation to dynamic changes in host plant quality. *Biol. J. Linn. Soc. 35*, 339-350.

PHYSIOLOGY

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GUSTATORY CODES IN LEPIDOPTEROUS LARVAE

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ABSTRACT

The sensory code for taste quality which largely mediates acceptance or rejection of food by lepidopterous larvae lies in the activity of a small number of neurones associated with their mouthparts. Various manifestations of the across-fibre patterning code are discussed and evaluated using data from *Spodoptera* and *Heliothis*. The mechanisms associated with gustatory coding are illustrated along with the types of behavioural assays used to calibrate and validate them.

Key words: gustation, across-fibre patterning, sensory code, neurone, gustatory receptor, central nervous system, deterrent, phagostimulant, styloconic sensillum, azadirachtin, nicotine hydrogen tartrate, sucrose, epipharingeal sensillum, *Canna glauca, Scutellaria tournifortii, Spodoptera litoralis, Spodoptera exempta, Manduca sexta, Spodoptera frugiperda, Heliothis virescens, Heliothis armigera,* wheat, neem, cabbage, tomato, sunflower, sorghum, salicin, alanine, canavanine, sodium chloride, strychnine.

Dethier stated in his concluding remarks at the Pau conference in 1986 that although intense efforts were being undertaken to relate taste receptor encoding with the reality of insect behaviour, "we shall not be able to relate sensory events to behaviour with complete confidence until we learn to place electrodes in the central nervous system (CNS) accurately". We have not been able to undertake this latter and most important step but we have, with the use of a range of compounds and plant extracts, been able to identify types of neural output from the peripheral gustatory sensilla that correlate with behavioural responses. As outlined by Dethier (1987), formidable problems remain in interpreting many of the responses we encounter, especially when there is a great deal of variability (Molen et al., 1985), not only between individuals within a population, but between successive stimulations of the same sensillum on a single insect.

Gustatory neural codes are the means by which the activity of the peripheral taste receptors, and therefore their analysis of the gustatory environment, is conveyed to the CNS by the electrical activity of these taste neurones. In practice, the taste receptors of insects are primary neurones, conveying information only by trains of action potentials, and communicating with the CNS by synapses in the suboesophageal ganglion. Students of neural coding have identified numerous variants of this type of neural activity as examples of specific codes. For example, Perkel and

Bullock (1968), identified 32 types of neural code; more conservatively, Dethier (1976) identified 5 codes in the gustatory system of flies.

A common feature of many such studies is the concept of across-fibre patterning formulated by Pfaffman (1941) and elaborated by Erickson (1963, 1982), which states that the neural message can only be read when the output of a number of receptors is considered at the same time. The basic tenets of the hypothesis are that the receptors have different but overlapping response spectra and that each stimulus identified generates a unique response spectrum across the receptors. This mechanism appears not to operate however, in cases where one or more receptor neurones show high sensitivity to a particular stimulus, in which case its presence is reported unambiguously, perhaps even exclusively, by that specialised neurone. Such a system is referred to as a labelled line and is often associated with the detection of compounds having high biological significance, such as potent feeding deterrents (Ma, 1972; Blaney et al., 1987) or key phagostimulants (Blom, 1978). In most cases, insects have to make the decision to feed or not to feed on the basis of neural information reporting a mixture of deterrents and phagostimulants, where the balance of the two types of information will be taken, along with internal factors, to determine the behavioural response.

In our attempts to interpret the code we intercept that flow of neural information to the CNS, identify its elements as best we can, and attempt to gain from it the same information which the insect receives. It would seem that our success is likely to be predicated upon the accuracy with which we identify the relevant aspects of the neural message on the one hand, and the behavioural response associated with it on the other hand. We must also accept that we may read the code accurately but in a manner different from that in which the insects reads it. To avoid this flaw it would be necessary to record from second order neurones in the suboesophageal ganglion so as to monitor the way in which the peripheral input is processed, and thereby to identify relevant, as opposed to spurious, methods of analysing the peripheral data. Advances have been made in this direction with the olfactory system of insects (Christensen and Hildebrand, 1987) but the correlation of activity in peripheral and second order gustatory neurones has not yet been reported.

The nature of sensory input that has been analysed varies with experimental circumstances. In some cases it has not been possible to be any more precise than to consider the total output from a multi-neuronal sensillum over a one second period. Even so, this relatively crude estimate of neural activity can be correlated closely with behavioural responses (Schoonhoven and Dethier, 1966; Schoonhoven, 1969; Blom, 1978; Blaney et al., 1987, 1988).

In other cases the activity or electrical characteristics of one or more neurones within a sensillum make them sufficiently prominent in the recorded message that they can be reliably identified and their activity correlated with behaviour (Ma, 1972; Blaney et al., 1987, 1988). Occasionally, it has been possible to identify the activity of all the neurones responding (Dethier, 1973).

A similarly wide range of behavioural responses have been used successfully to validate interpretations of the neural code. Recently, we have stimulated the maxil-

lary styloconic sensilla of sixth stadium larvae of *Spodoptera littoralis* (Boisduval) with a range of concentrations (10^{-4} M to 10^{-8} M) of azadirachtin, and correlated aspects of the neural response with various behavioural assays, in which the insects were offered plant material coated with the relevant concentrations of azadirachtin. The total output over one second, from both the lateral and medial sensilla, correlates linearly with three very different behavioural assays: the amount of faecal material produced over a 24 h period ($R^2 = 0.854$, $p<0.00$, $n = 50$), the total amount eaten over a 8 h period ($R^2 = 0.8792$, $p<0.001$, $n = 50$) and the duration of the first meal ($R^2 = 0.7731$, $p<0.001$, $n = 50$).

First meal duration correlates exponentially with the ouput from the medial sensillum alone ($R^2 = 0.8017$, $p<0.001$, $n = 50$) and with the activity of the most vigorously responding neurone in the medial sensillum ($R^2 = 0.8421$, $p<0.001$). This degree of correlation across a range of neural and behavioural assays occurs when the response is dominated by the activity of a single neurone which strongly affects the behaviour of the insect over a lengthy period. Such a response is typical of a labelled line.

Although such a labelled line constitutes a very robust stimulus-response system it is not immutable. In particular, the relationship can be modified by the dietary experience of the insect, as has been described in "induction" (Jermy et al., 1968; Schoonhoven, 1969; Ma, 1972; Staedler and Hanson, 1976; Blaney and Simmonds, 1984; Jermy, 1987). Similarly, the responses of insects vary with age in the stadium, time of day and diet (Blaney et al., 1987; Schoonhoven et al., 1987; Blaney and Simmonds, 1987). This raises the interesting question of whether the code itself varies or whether it is the nature of the information encoded by the peripheral receptors that changes. We have found that in larvae of *Spodoptera littoralis*, induced by exposure to nicotine hydrogen tartrate (NHT) in the diet at levels that reduce feeding by acting as a deterrent, the subsequent increased intake of treated food is associated with decresed sensitivity of a receptor responsive to NHT (Blaney and Simmonds, 1983, 1987). Thus, the indications are that the code is not altered, but that the behavioural change, which occurs over several days, is brought about by a change in peripheral sensitivity. The results of this experiment give indirect evidence that we are reading the gustatory code in the same way that the insect does.

Another form of mutability of a labelled line system depends for its expression on the circumstances of the investigation. Larvae of *Spodoptera exempta* (Walker) have a neurone in the medial styloconic sensilla which is sensitive to sucrose, and another identifiably different neurone which is sensitive to azadirachtin. When either of these stimulants is applied separately, the responses of the relevant neurones can readily be identified and they have all the characteristics of labelled lines. When the two stimulants are applied together, the responses of the two neurones are separate and independent of each other. If a similar protocol is applied with larvae of *Spodoptera littoralis* it is possible to identify neurones sensitive to sucrose and azadirachtin respectively, giving dose-response relationships, provided the two stimulants are applied separately. When they are applied together, there is an obvious interaction affecting the response of both neurones (Simmonds and Blaney, 1984). The

effect of this interaction is that increasing the concentration of either one of the stimulants causes a decrease in the response to the other. Thus, with *Spodoptera littoralis* the apparent labelled lines for sucrose and azadirachtin only act as such, in some experimental situations. This type of interaction has been shown in a number of other polyphagous species (Simmonds and Blaney, 1984).

We have referred earlier to the desirability of validating our interpretation of the sensory message by considering the behavioural response elicited by it. It is clearly important to choose appropriate behavioural assays, but it is equally important to identify a sensory message which can be expected to be solely, or largely responsible for eliciting the behaviour. The maxillary styloconic sensilla of lepidopterous larvae are an ideal system in this context. Ablation experiments (Hanson and Dethier, 1973) have shown that chemosensory discrimination still occurs when all sensilla except the styloconica and the epipharyngeal sensilla have been destroyed, and separate experiments (de Boer et al., 1977) have shown that the epipharyngeal sensilla are involved principally with swallowing.

Caterpillars have two lateral and two medial styloconic sensilla and each sensillum has four chemosensory neurones, thus the critical neural message for food selection is contained in the activity of only 16 neurones. The most commonly applied technique of recording the activity of these neurones, the tip-recording method (Hodgson et al., 1955), results in all the neural activity in one sensillum being recorded at the

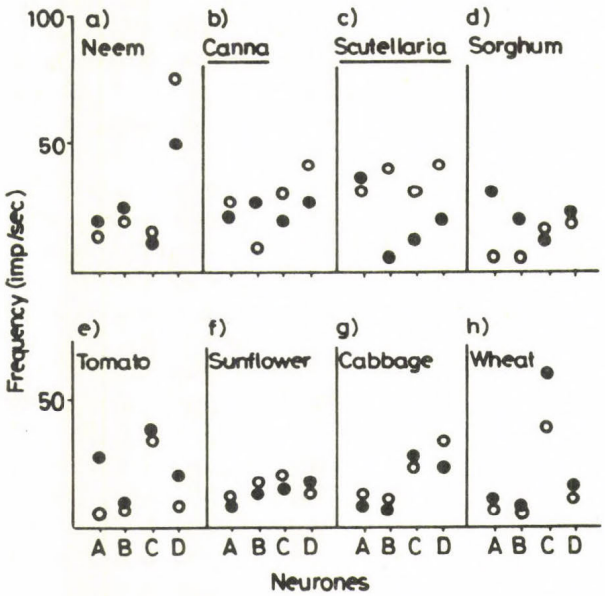

Fig. 1 (abcdefg). Responses (impulses in first second) of all four neurones in lateral (O) and medial (●) sensilla of *Spodoptera littoralis* to stimulation with saps from 8 species of plant. (n = 15)

same time. Commonly, and particularly with complex stimulating solutions such as plant saps, all four neurones within the sensillum may be active at the same time. To investigate the neural code it is desirable to be able to identify the activity of individual neurones accurately. We rely not only on the amplitude of the action potentials as a discriminating characteristic but also on other properties, such as their shape and slope. This interpretation is made easier and more secure by the use of specially designed computer programmes (Hanson, 1983; Frazier and Hanson, 1986).

In reality, insects assessing potential plants for their suitability will almost always have their taste receptors stimulated by a complex mixture of natural compounds. Dethier and Crnjar (1982) suggested that larvae of *Manduca sexta* (Johanssen) were able to discriminate between the saps of three plants using across-fibre patterning to convey the gustatory code. The important part of the code was the relative rate of firing and the variance of all the sensory neurones that could be identified as active in the styloconic sensilla during the tonic phase of the response. Our work, and the earlier work of Dethier and Kuch (1971) and Dethier (1973), has shown that the responses to plant saps are multi-neuronal. There is no single pattern of response that signals acceptance nor, conversely, does any single pattern signal rejection.

We have stimulated the styloconic sensilla of four species of lepidopterous larvae *(Spodoptera littoralis, S. frugiperda* (J. E. Smith), *Heliothis virescens* (Fabricius) and *H. armigera* (Hubner) with freshly expressed saps of eight potential host plants, ranging from acceptable (seedling wheat, chinese cabbage, tomato (Strain B), sunflower, sorghum) to unacceptable (neem, *Canna glauca, Scutellaria tournifortii)* for the four species of lepidopterans, whose preferences in this respect are somewhat similar.

The firing rates of all four neurones in both medial and lateral sensilla in response to the saps for *S. littoralis* are shown in Fig. 1. We have designated the neurones A, B, C and D in order to avoid teleological implications of the neural specificity associated with such terms as "sugar receptor" or "sugar-best receptor". Nevertheless, the neurones can be characterised on the basis of their response spectra to a commonly used range of test compounds applied individually. Thus, while a neurone may respond well to stimulations with sugars it cannot be guaranteed, without exhaustive testing, that any particular sugar is the best stimulus for that neurone. It is less restrictive therefore, to associate each neurone with a reference compound to which it responds reliably across a population of insects. The reference compounds for the neurones in the styloconic sensilla of the four species studied here are: medial sensillum neurone A = salicin, B = alanine, C = sucrose, D = canavanine; lateral sensillum neurone A = sodium chloride, B = alanine, C = sucrose, D = strychnine.

It is clear from Fig. 1abc that overall, in response to the unacceptable saps, the rate of firing of neurones A and D is higher than that of B and C in both lateral and medial sensilla. Further, whilst it has been previously reported that, in some caterpillars, the main message for "deterrence" has been carried by the medial sensillum (Ma, 1972; Blaney et al., 1987, 1988) the present experiments, using plant saps, reveal that there seems to be almost equal contribution of information from both sensilla.

When the favoured plants are considered (Fig. 1defgh) a different picture emerges. The total firing rates are lower than those elicited by less favoured plants (Fig.1abc) and, overall, the firing rates of B and C are higher than those of A and D.

Similar responses from the other three species to the most extreme cases, a rejected plant neem and an accepted plant wheat, are shown in Fig. 2. There is a marked congruence of response patterns with only the response of *H. armigera* (Hübner) to neem being at variance with the others. Thus, it would seem from inspection of these figures that the message signalling acceptance or deterrence hinges on the balance of A + D against B + C, as illustrated for *S. littoralis* in Fig. 3. This model of the gustatory code places wheat at one end of the response range

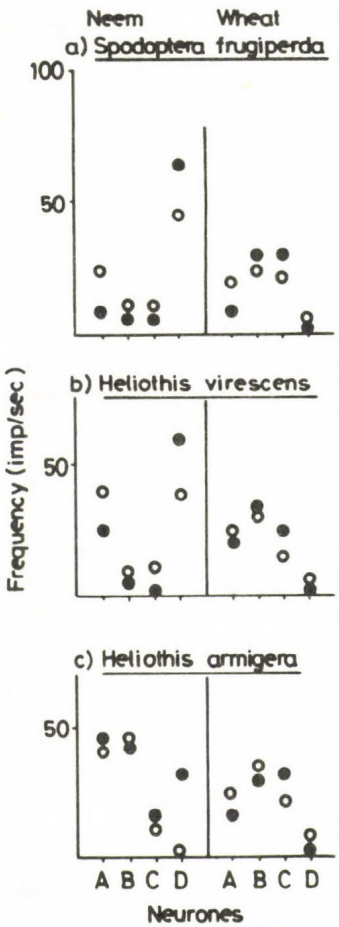

Fig. 2 Responses (impulses in first second) of all four neurones in lateral (O) and medial sensilla (●) of *Spodoptera frugiperda, Heliothis virescens* and *Heliothis armigera* to stimulation with saps from neem and wheat.

and neem at the other. However, it does not separate the other plants as clearly as they are distinguished behaviourally.

Evidently the gustatory code actually used by the insects is more sophisticated than this. The model illustrated in Fig. 3 takes account of the relative responses of neurones but ignores their actual rate of firing. When the actual firing rate of the "deterrent" neurones is considered (Fig. 4), the separation is more complete and

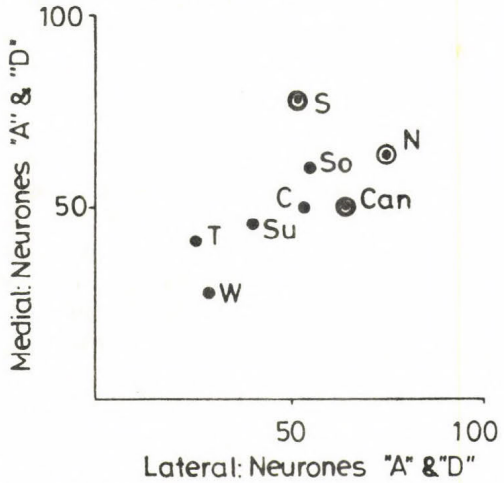

Fig. 3 Responses of neurones A and D, as a proportion of total impulses in lateral and medial sensilla respectively, of *Spodoptera littoralis* to stimulation with saps from the eight plants tested (N = neem, Can = *Canna*, S = *Scutellaria tournifortii*, So = sorghum, T = tomato (strain B), Su = sunflower, C = chinese cabbage, W = wheat).

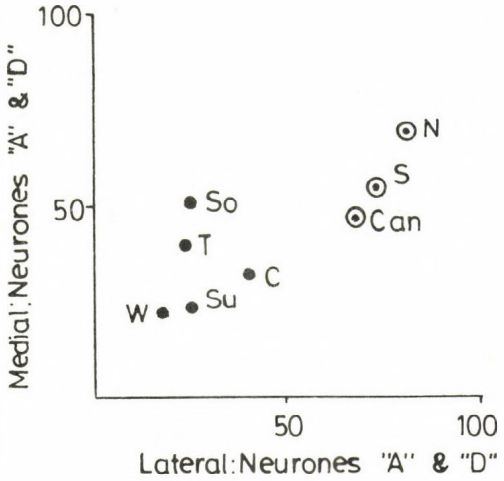

Fig. 4 Actual firing rate (impulses in first second) of neurones A and D in lateral and medial sensilla of *Spodoptera littoralis* to stimulation with saps from the eight plants tested (see Fig. 3 for details).

accords well with the behaviour. This last approach, however, ignores the contribution of the "phagostimulant" neurones B and C to the decision-making process. The relative importance of the two groups of neurones may be assessed from Fig. 5, in which the responses to the eight plants are considered. The plants fall into two groups, the acceptable and the unacceptable. There is little difference in the phagostimulatory input from neurones B and C between the two groups of plants. Conversely, there is a marked difference in deterrent input from neurones A and D, which clearly separates the two groups. This result accords well with the earlier concept that plants have broadly similar phagostimulatory properties (Thorsteinson, 1960) and that host selection is based on the profiles of deterrent compounds found in non-host plants (Jermy, 1984).

These concepts apply to all four species (Fig. 5). Another aspect of coding illustrated by the data in Fig. 5 is that a much greater variability in response is associated with plants that are rejected, in comparison with those which are accepted.

Rejection of potential food material can occur at one or more of the sequential steps involved in food selection behaviour. We have found that different neural mechanisms are associated with rejection at different stages in the sequence. Typically, the larva initiates its investigation of food material with maxillary palpation.

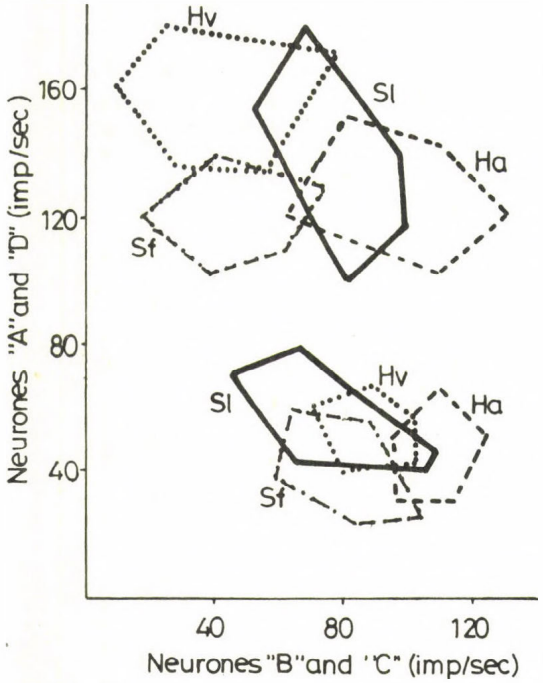

Fig. 5 Actual firing rate (impulses in first second) of neurones B and C, and neurones A and D, in lateral and medial sensilla of all four species to stimulation with saps from eight plants tested. Profiles enclose the standard errors of the mean in each case. (Sl = *Spodoptera littoralis*, Sf = *Spodoptera frugiperda*, Hv = *Heliothis virescens*, Ha = *Heliothis armigera)*

Compounds capable of stopping the investigation at this early stage include high concentrations of quinine or tomatine, which causes "bursting", a brief period of very rapid firing in one or more neurones (Sturchkow, 1959; Ma, 1977; Dethier, 1980; Schoonhoven, 1982; Mitchell and Sutcliffe, 1984).

If however, the insect is not deterred at palpation it will go on to take a bite. The sequence may be stopped at this stage by bursting, or by a high firing rate in a deterrent neurone (e.g. azadirachtin). The next step in the sequence, sampling a small piece of food material, can be distrupted by compounds, such as warburganal, showing latency in their effect on deterrent neurones. If no such disruption occurs, prolonged feeding may commence. However, even if continuous feeding has started it may be curtailed by a deterrent activating a labelled line (e.g. salicin) or contributing to the across-fibre pattern of input, as occurs, with the alkaloid 2,5,dihydroxymethyl-3,4,dihydroxypyrrolidine (DMDP).

In summary, our investigations lead us to conclude that gustatory coding in lepidopterous larvae is dominated by across-fibre patterning. We have shown that labelled lines may or may not operate, depending on the circumstances, and would suggest therefore that the labelled line is merely one end of the across-fibre spectrum. We also suggest that other so called mechanisms of coding, variously referred to as "disruption" including bursting and blocking, occur where the normal coding has been interfered with, and the message is conveyed by a malfunction.

REFERENCES

Blaney, W. M. and M. S. J. Simmonds (1983): *Electrophysiological Activity in Insects in Response to Antifeedants.* TDRI Miscell. Publ., London.

Blaney, W. M. and M. S. J. Simmonds (1984): Experience of chemicals alters the taste sensitivity of lepidopterous larvae. *Chem. Senses 8*, 245.

Blaney, W. M. and M. S. J. Simmonds (1987): Experience: A modifier of neural and behavioural sensitivity. In: V. Labeyrie, G. Fabres and D. Lachaise (eds), *Insects-Plants, Proc. 6th Inter. Symp. Insect-Plants Relationships (Pau 1986)*, Junk, Dordrecht, 237-241.

Blaney, W. M., L. M. Schoonhoven, and M. S. J. Simmonds (1986): Sensitivity variations in insect chemoreceptors; a review. *Experientia 42*, 13-19.

Blaney, W. M., M. S. J. Simmonds, S. V. Ley, S. V. and R. B. Katz (1987): An electrophysiological and behavioural study of insect antifeedant properties of natural and synthetic drimane-related compouds. *Physiol. Entomol. 12*, 281-291.

Blaney, W. M., M. S. J. Simmonds, S. V. Ley, and P. S. Jones (1988): Insect antifeedants: a behavioural and electrophysiological investigation of natural and synthetically derived clerodane diterpenoids. *Entomol. exp. appl. 46*, 267-274.

Blom, F. (1978): Sensory activity and food intake: a study of input-output relationships in two phytophagous insects. *Neths. J. Zool. 28*, 277-340.

Christensen, T. A. and J. G. Hildebrand (1987): Functions, organization, and physiology of the olfactory pathways in the lepidopteran brain. In: A. P. Gupta, (ed).

Arthropod Brain its Evolution, Development, Structure, and Functions. J. Wiley and Sons, New York. 457-484.

de Boer, G., V. G. Dethier, and L. M. Schoonhoven (1977): Chemoreceptors in the preoral cavity of the tobacco hornworm, *Manduca sexta*, and their possible function in feeding behaviour. *Entomol. exp. appl. 21*, 287-298.

Dethier, V. G. (1973): Electrophysiological studies of gustation in lepidopterous larvae. II. Taste spectra in relation to food-plant discrimination. *J. Comp. Physiol. 82*, 103-134.

Dethier, V. G. (1976): *The Hungry Fly: A Physiological Study of the Behaviour Associated with Feeding.* Harvard University Press, Cambridge, MA.

Dethier, V. G. (1980): Evolution of receptor sensitivity ot secondary plant substances with special reference to deterrents. *Am. Nat. 115*, 45-66.

Dethier, V. G. (1987): Concluding Remarks. In: V. Labeyrie, G. Fabres and D. Lachaise (eds), *Insects-Plants, Proc. 6th Inter. Symp. Insect-Plant Relationships (Pau 1986)*, Junk, Dordrecht. 429-435.

Dethier, V. G. and R. M. Crnjar (1982): Candidate codes in the gustatory system of caterpillars. *J. Gen. Physiol. 79*, 543-569.

Dethiers, V. G. and J. H. Kuch (1971): Electrophysiological studies of gustation in lepidopterous larvae. I. Comparative sensitivity to sugars, amino acids, and glycosides. *Z. Vgl. Physiol. 72*, 543-569.

Erickson, R. P. (1963): Sensory neural patterns and gustation. In: Y. Zotterman (ed), *Olfaction and Taste.* Pergamon Press, London. 205-213.

Erickson, R. P. (1982): The across-fiber pattern theory: An organizing principle for molar neural function. *Contrib. Sensory Physiol. 6*, 205-213.

Frazier, J. L. and F. E. Hanson (1986): Electrophysiological recording and analysis of insect chemosensory responses. In: J. R. Miller, and T. A. Miller (eds), *Insect-Plant Interactions*, Springer-Verlag, New York. 285-330.

Hanson, F. E. (1983): The behavioural and neurophysiological basis of food-plant selection by lepidopterous larvae. In: S. Ahmad, (ed.) *Herbivorous Insects, Host-seeking Behavior and Mechanisms*, Academic Press, New York. 3-23.

Hanson, F. E. and V. G. Dethier (1973): Role of gustation and olfaction in food plant discrimination in the tobacco hornworm, *Manduca sexta. J. Insect Physiol. 19*, 1019-1034.

Hodgson, E. S., J. Y. Lettvin, and K. D. Roeder (1955): Physiology of a primary chemoreceptor unit. *Science 122*, 417-418.

Jermy, T. (1984): Evolution of insect/host plant relationships. *Am. Nat. 124*, 609-630.

Jermy, T. (1987): The role of experience in host selection of phytophagous insects. In: R. F. Chapman, E. A. Bernays and J. G. Stoffolano, Jr. (eds), *Perspectives in Chemoreception and Behavior*, Springer-Verlag, New York. 142-157.

Jermy, T., F. E. Hanson, and V. G. Dethier (1968): Induction of specific food preference in lepidopterous larvae. *Entomol. exp. appl. 11*, 211-230.

Ma, W.-C. (1972): Dynamics of feeding responses in *Pieris brassicae* Linn. as a function of chemosensory input: a behavioural, ultrastructural and electrophysiological study. *Meded. Landbouwhogesch. Wageningen 72-11.*

Ma, W-C. (1977): Alterations of chemoreceptor function in armyworm larvae (*Spodoptera exempta)* by a plant derived sesquiterpenoid and by sulfhydryl reagents. *Physiol. Entomol. 2*, 199-207.

Mitchell, B. K. and J. F. Sutcliffe (1984): Sensory inhibition as a mechanism of feeding deterrence: effects of three alkaloids on leaf beetle feeding. *Physiol. Entomol. 9*, 57-64.

Molen, J. N. van der, L. J. Nederstigt, and C. L. Veenman (1985): Variability in blowfly taste responses. *J. Comp. Physiol. A 157*, 211-221.

Perkel, D. H and T. H. Bullock (1968): Neural coding. *Neurosciences Res. Program Bull. 6*, 221-258.

Pfaffmann, C. (1941): Gustatory afferent impulses. *J. Cell Comp. Physiol. 17*, 243-258.

Schoonhoven, L. M. (1969): Sensivity changes in some insect chemoreceptors and their effect on food selection behaviour. *Proc. K. Ned. Akad. Wet. C 72*, 491-498.

Schoonhoven, L. M. (1982): Biological aspects of antifeedants. *Entomol. exp. appl. 31*, 57-69.

Schoonhoven, L. M. and V. G. Dethier (1966): Sensory aspects of host-plant discrimination by lepidopterous larvae. *Arch. Neerl. Zool. 16*, 497-530.

Schoonhoven, L. M., W. M. Blaney, and M. S. J. Simmond (1987): Inconstancies of chemoreceptor sensitivities. In: V. Labeyrie, G. Fabres and D. Lachaise (eds), *Insects-Plants, Proc. 6th Inter. Symp. Insect-Plant Relationships (Pau 1986),* Junk, Dordrecht. 141-145.

Simmonds, M. S. J. and W. M. Blaney (1984): Some effects of azadirachtin on lepidopterous larvae. In: H. Schmutterer, and K. R. S. Asher (eds), *Proc. 2nd. Int. Neem Conf.* GTZ GmbH, Eschborn. 163-180.

Staedler, E. and F. E. Hanson (1976): Influence of induction of host preference on chemoreception of *Manduca sexta. Physiol. Entomol. 3*, 121-133.

Stürckow, B. (1959): Über den Geshmackssinn und Tastsinn von *Leptinotarsa decemlineata* Say (Chrysomelidae). *Z. Vergl. Physiol. 42*, 255-302.

Thorsteinson, A. J. (1960): Host selection in phytophagous insects. *Ann. Rev. Entomol. 5*, 193-218.

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SENSORY CODING IN *MANDUCA SEXTA* FOR DETERRENCE BY A NON-HOST PLANT, *CANNA GENERALIS*

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ABSTRACT

Antifeedant phytochemicals have a profoundly negative effect on the food selection of many species of caterpillars. It has been suggested that rejection is mediated by stimulation of "deterrent cells", inhibition of "acceptance cells", or more complex codes. Evidence is presented here for the first of these. The lateral styloconicum contains a deterrent cell which is highly sensitive to a variety of antifeedants such as caffeine, aristolochic acid, and salicin. It is the medial styloconica, however, that is responsible for deterring feeding on the unacceptable non-host plant, *Canna generalis*.

The deterrent cell in the medial styloconica has not been characterized previously. Electrophysiological studies show that certain extracts of *Canna* elicit a vigorous response from predominantly one cell. Behavioral experiments show that these extracts are rejected by the insects. Partially purified fractions of the extract stimulate only this cell. A characteristic of the recorded spike is that it increases in peak-to-peak voltage with increasing stimulus concentration. These observations were made in both *Manduca sexta* and *quinquemaculata*.

A model of deterrence by the medial deterrent cell will be discussed and contrasted with other recorded responses that are candidate deterrent codes.

Key words: chemoreception, gustation, receptor coding, deterrency, feeding behavior, *Manduca sexta*, Lepidoptera.

INTRODUCTION

The acceptability of potential foodplants for the tobacco hornworm, *Manduca sexta*, is graded and ranges from highly acceptable host plants to completely unacceptable non-host plants (deBoer and Hanson, 1984.) The sensory systems detect and code the essential qualities of each encountered plant, thereby providing the central nervous system (CNS) with sufficient information to make a decision whether or not to feed on this plant. The most deterrent plants are likely to be identified unambiguously and the information emphatically communicated to the CNS so the rejection behavior can be manifest immediately and completely. For example, the canna lilly, *Canna generalis*, is so unacceptable to the tobacco hornworm that the insect will starve to death rather than eat it. However, ablation of the chemosensory systems will result in substantial feeding on this plant (deBoer and Hanson, 1987a). The plant does not appear to be acutely toxic, since those ablated larvae that consume

large amounts will develop into normal size adults provided they also ingest sufficient host plant material. Thus the sensory system is of paramount importance in controlling feeding behavior.

Of all the sensory systems possibly responsible for rejection of unacceptable plants, the gustatory system is clearly the most important. Waldbauer and Fraenkel (1961) showed that removal of the maxillary sensilla styloconica of the tobacco hornworm resulted in widening of its host range. The chemosensory ablation studies referred to above (deBoer and Hanson, 1987a) showed that canna was completely rejected if only the medial styloconica (unilateral or bilateral) remained. The same was true if only the epipharyngeal receptors were present. The other chemosensory organs (antennae, maxillary palpi and lateral styloconica) were unimportant for this discrimination and did not contribute to deterrence of feeding on canna. Accordingly, this study will focus on deterrence coding by the medial styloconica in response to the class of unacceptable plants represented by *C. generalis*.

Understanding sensory coding requires quantitative information about behavior correlated with the sensory input that caused that behavior. The medial styloconica represent such a possibility, based on the ablation experiments of deBoer and Hanson (1987a) discussed above. Since the animals rejected canna when the medial styloconica were the only chemosensory organ present, it can be concluded that the sensory inputs from the medial cause rejection. Further study of this input requires an active extract of the plant suitable for use in the stimulating pipette while recording from the sensilla using electrophysiological techniques. Therefore, this paper will report on obtaining extracts of canna, testing them behaviorally to find one that is deterrent, and recording sensory activity in the medial styloconica elicited by the active extract.

Medial styloconica contain 4 sensory dendrites that are likely chemosensory, and another which may be both mechanosensitive and chemosensitive (Zacharuk, personal communication). Electrophysiological recordings demonstrated the presence of a mechanosensitive cell (Hanson, 1970). Schoonhoven (1969, 1982) found a glucose-sensitive cell and an inositol-sensitive cell, both of which are probably quite specific (Frazier, 1986). The molecular receptor site for the former has been determined (Lam and Frazier, personal communication). Recordings also show that one or more cells respond to salts, albeit rather poorly and not in the dose-dependent manner typical of the other chemosensory cells. Completing the sensory complement is a cell that Schoonhoven (1969) referred to as a "deterrent" cell sensitive to some alkaloids. The last mentioned cell, the function of which has not been examined, is clearly the cell of interest for any investigation of deterrence coding in the medial styloconica and accordingly will be considered in some detail.

MATERIALS AND METHODS

Manduca sexta eggs were obtained from the U.S.D.A. in Beltsville, MD, and reared on diet (BioServ, Frenchtown, NJ) in 16:8 LD cycle at ca. 27°C.

Foliage of *Canna generalis* was weighed, blended, and serially extracted with the polarity series: water (90°C), ethanol (60°C), diethyl ether or ethyl acetate, and chloroform or hexane. After establishing that ethanol is the most effective solvent, the extraction procedure was simplified by directly blending fresh foliage with hot ethanol. Extracts were stored in the dark at -10°. This crude extract was used for the early behavioral bioassays.

Improvements of the above extraction process, including "defatting" the crude extract with hexane in a separatory funnel, resulted in a cleaner extract that was used for some behavioral and electrophysiological assays. This extract was further fractionated by reverse phase column chromatography using a procedure that separates compounds on the basis of minor differences in polarity. Five fractions were obtained by elution with water, water:methanol (1:1), and three successive methanol aliquots (Peterson, Hanson, and Warthen, unpublished).

Extracts were bioassayed using the disc test of Jermy, *et al.* (1968) as modified by deBoer and Hanson (1988). Briefly, 6 discs cut from glass fiber filter paper (Wattman GF/A) were placed around the perimeter of a circular dish ca. 10 cm in diam. Discs were held on pins 1 cm above the bottom of the dish. Extracts or fractions were spotted on alternate discs, with solvent on control discs. After evaporation of the solvent, discs were wetted with 0.1 ml water. A recently molted, unfed fifth instar larva was placed in the center of each test chamber and allowed to feed until 50% of the most eaten category was consumed at which time the area consumed of each disc was visually estimated. Normally, 20 animals were tested at each concentration. Tests were conducted in the dark at ca. 27°. Results were calculated as inhibition index: (control-test)/(control+test)x(100).

Sensory responses were recorded using standard electrophysiological techniques (Hanson, 1970) with the active Ag-AgCl electrode in contact with recording/stimulating pipette containing the ethanolic extract or fraction made up in a final concentration of ca. 10% ethanol and 0.1 M NaCl. Controls were 0.1 M Nacl or 0.1 M NaCl in 10% ethanol. Each trial lasted ca. 3 sec and intertrial intervals were ca. 3 min.

RESULTS

Behavioral assays. The serial extracts of the foliage of canna macerated in the blender were tested behaviorally. The hot water extract was stimulatory, the hot ethanol extract was deterrent, the diethyl ether or ethyl acetate extract was neutral and the chloroform or hexane extract was stimulatory. Based on this information, an extract of fresh foliage blended with hot ethanol was used as the stock solution for a dose-response curve of deterrency (Fig. 1). This extract was clearly deterrent: an inhibition index of ca. 75% was attained at the highest concentration (ca. 1 g FW/ml), calculated to be approximately 0.4 times (for extract #4 in Fig. 1) and 0.7 times (for extract # 12) the concentration of the ethanol-soluble components of the fresh leaves. This calculation assumed that the extraction procedure would be 100%

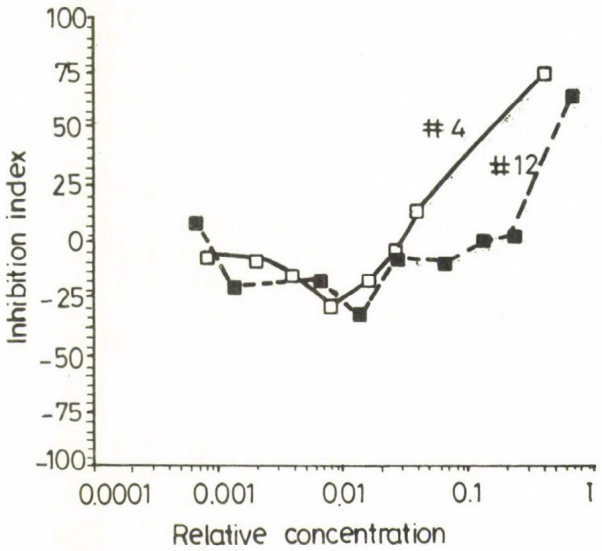

Fig. 1 Behavioral deterrency of the crude ethanolic extract of *Canna generalis* for *Manduca sexta* in the disc test. The inhibition index (control-test)/(control + test)x(100) increases with concentration in two different extracts (#4 and #12). A relative concentration of 1.0 means that the ethanol-extractable compounds from a given area of foliage are spotted on the equivalent area of filter paper discs.

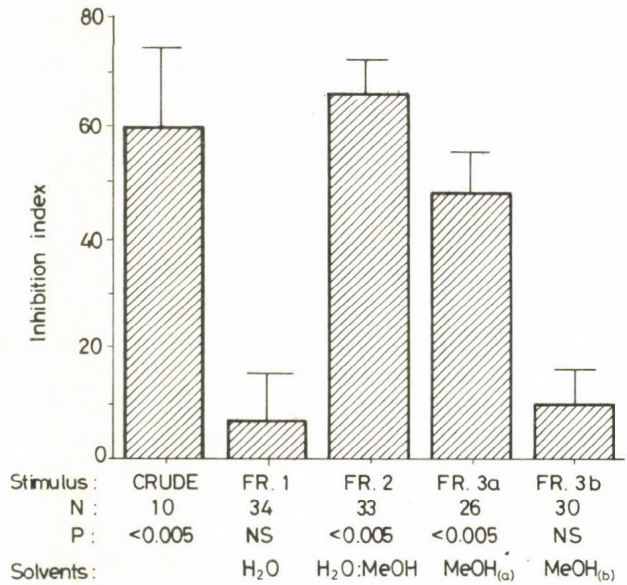

Fig. 2 Behavioral deterrency of the fractions obtained by reverse phase column chromatography. The third methanol fraction (FR. 3c) was inactive and is not shown. Inhibition index was calculated as in Fig. 1.

efficient, which is highly unlikely. If not defatted prior to testing, the diluted extract was slightly stimulatory (Fig. 1).

The fractionation of the active extract clearly isolated the deterrency in the water:methanol and first methanol fractions (Fig. 2). The degree of deterrence was comparable to that of the crude extract starting material.

Electrophysiological responses of medial sensilla styloconica. Stimulation of the medial styloconica with a series dilution of the defatted crude extract elicited responses from primarily one cell (Fig. 3). Spike frequency clearly increases with concentration of the extract. It is also clear that the spike amplitude is variable: it increases during the first 100 msec of a vigorous response, and it also increases with concentration.

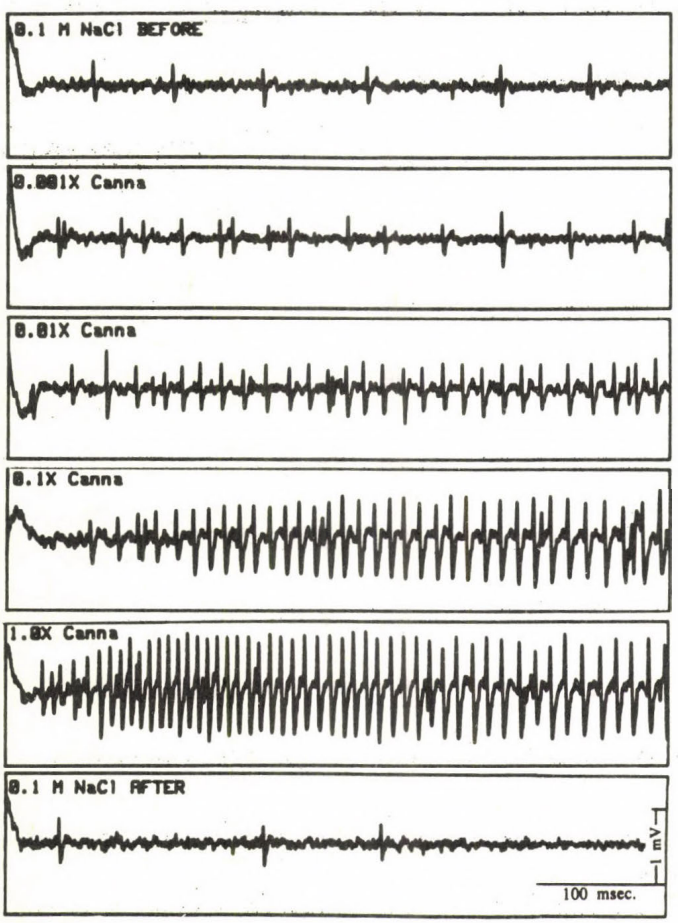

Fig. 3 Sensory responses of the medial styloconica to dilutions of crude extract in 0.1 M NaCl and ca. 10% ethanol. The controls are 0.1 M NaCl + 10% Ethanol. The first 500 msec of a 3 sec trial is shown in each trace.

The chromatographic fractions of the extract activated one cell in the medial styloconica, apparently the same cell that predominates the response to the crude extract (Fig. 4). Only the behaviorally active fractions elicited responses.

The cell active in response to canna extracts is different than either of the two previously identified cells, namely the "glucose cell" and the "inositol cell". This was ascertained from responses to mixtures of stimuli. Combining either of these compounds with the canna extract elicited different responses than did the compound or extract alone. Each characteristic spike shape could be recognized in the mixed response, spike frequencies in response to mixtures were additive, and algebraic additions of the spike amplitudes resulted in an uneven and ragged spike train. These are characteristics of multiple cells responding independently.

Other secondary plant compounds were tested in an attempt to determine the response spectrum of this sensory cell. One compound that is deterrent for many insects, the triterpene limonin, elicits a response from the medial sensillum similar to that of the canna extract (Fig. 5). Other compounds that are often cited as deterrents for the tobacco hornworm, such as nicotine, salicin, and caffeine do not

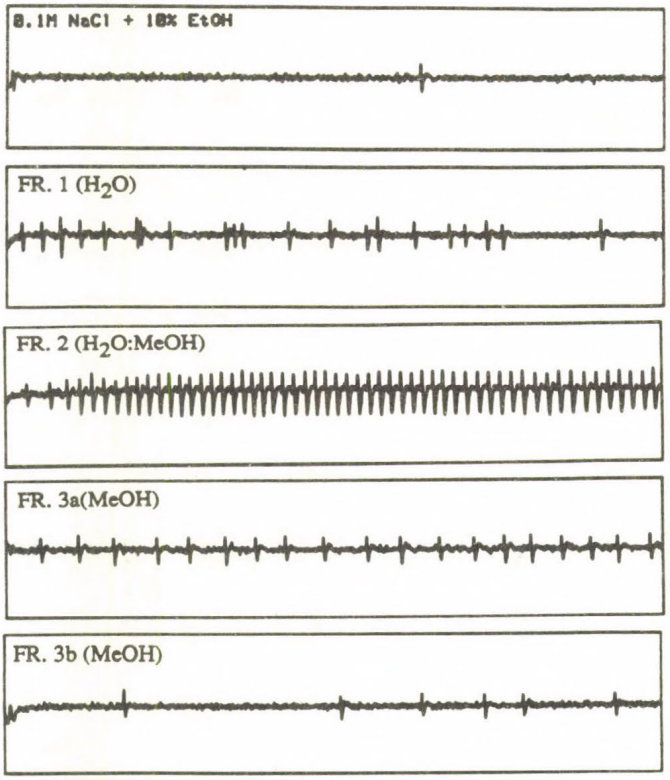

Fig. 4 Sensory responses of the medial styloconica to the same fractions tested behaviorally in Fig. 2. The first 500 msec of a 3 sec trial is shown in each trace.

stimulate the medial but do stimulate the lateral sensillum. A solanaceous glycoalkaloid, tomatine, causes bursting (at 1 mM) in the medial and lateral after continuous exposure for ca. 30 seconds, but does not appear to be behaviorally deterrent to the tobacco hornworm at natural concentrations (ca. 1 mM) and may even be slightly stimulatory (deBoer and Hanson, 1987b).

Electrophysiological responses of lateral sensilla styloconica. In contrast to the response of the medial, the lateral sensillum responds to canna extract with a more complex spike train comprised of action potentials from three or four cells. These spikes could not be reliably classified into categories associated with identified cells. The summed response of all spikes was dose dependent. The lateral response was not pursued further since this sensillum could not be associated with behavioral rejection of canna (deBoer and Hanson, 1987a).

DISCUSSION

We conclude that the sensory coding for rejection of unacceptable plants such as canna includes the vigorous response of a chemosensory cell in the medial styloconicum (the medial "deterrent cell"). In response to concentrations of the canna extract which elicited a threshold behavioral deterrence, the peak instantaneous frequency of this cell was ca. 50 spikes/sec. Higher concentrations elicited peak instantaneous frequencies of up to 200 spikes/sec. We do not, however, know that the animal uses instantaneous frequency as an input parameter in its decision process; perhaps it

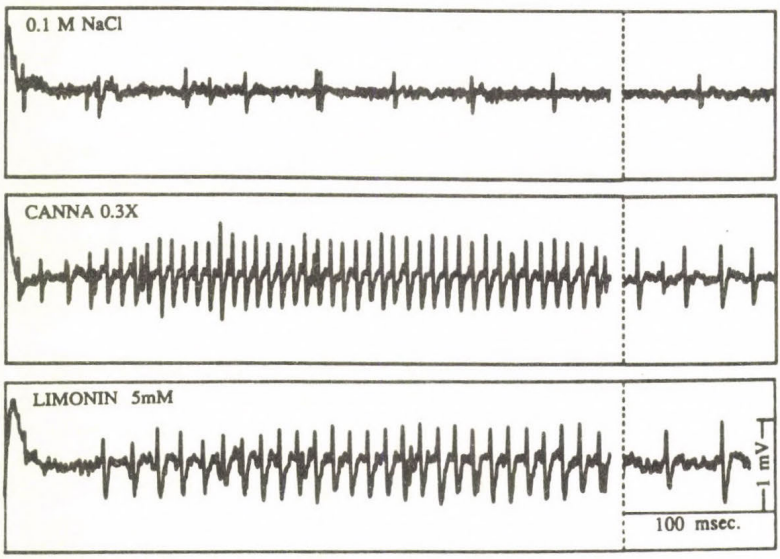

Fig. 5 Sensory responses of the medial styloconica to crude canna ethanol extract calculated to be 0.3 X the normal concentration of ethanol-soluble phytochemicals (ca. 1 g/ml) in 0.1 M NaCl and ca. 10% ethanol and to 5 mM limonin in unknown electrolyte concentration. The control is 0.1 M NaCl. The first 400 msec and the last 100 msec of a 3 sec trial are shown in each trace.

integrates all the input during the first second, for example, in which case the appropriate data would be ca. 30 spikes in the first second at threshold concentration.

In addition to that of the medial styloconica, other inputs must also contribute to the overall information base used by the CNS in its decisions. Certainly the epipharyngeal receptors studied by deBoer, *et al.* (1977) contribute information as well. The work of deBoer and Hanson (1987a) indicates that either the medial deterrent cell or the epipharyngeal input is sufficient to block feeding on canna. These two sensilla probably contribute sensory input in a hierarchical manner, since in the sequence of feeding events, contact with the phytochemicals by the styloconica would occur prior to that by the epipharyngeal sensilla.

Not all the sensory input from rejected plants is "deterrent". We know that the lateral styloconicum is moderately stimulated by the crude canna extract, but the sensory ablation study of deBoer and Hanson (1987a) indicates that this sensillum either does not contribute substantial amounts of deterrent information or else it is ignored. We also know that non-polar fractions of the crude extract are clearly feeding stimulatory: perhaps these are detected and mediated by the lateral styloconicum. This input may tend to counteract that from the deterrent cells, and therefore a complete investigation of sensory coding of rejected plants should continue to look at these inputs. Similar statements may also apply to the maxillary palpi which probably also have gustatory sensilla.

The isolation of the deterrent fraction in an ethanol extract and subsequently in the reverse phase chromatographic fractions indicates that the deterrent phytochemicals are relatively polar. This is in contrast to stimulatory extracts obtained with non-polar solvents from the surfaces of both host and non-host plant foliage (deBoer and Hanson, 1988). Another difference is that the deterrent extract seems to be fragile and must be obtained by procedures that inactivate degrading enzymes, whereas the stimulants are easily extracted and relatively robust. Further analysis of these fractions will hopefully permit chemical identification of the active principles.

Three unique characteristics of the response of the deterrent cell are its relatively long latency, slowly increasing spike frequency, and increase in spike amplitude with concentration of the extract. These characteristics have also been reported for other deterrent cells (Frazier, Simmonds, personal communications). In contrast, the glucose and inositol cells in the medial styloconicum have a five or ten-fold shorter latency and rise in spike frequency, and changes in spike amplitude do not appear to be directly related to concentration.

Thus our results support the hypothesis that a medial "deterrent cell" is important in inhibiting feeding on non-host plants. Integration of these results with the ablation studies of deBoer and Hanson (1987a) leads to the conclusion that this one cell, bilaterally or unilaterally, can completely block feeding behavior. This cell has a different response spectrum from that of the lateral deterrent cell; accordingly, these two cells provide the animal with the capability of detecting a greater range of deterrent phytochemicals than could be achieved with input from one cell alone.

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REFERENCES

deBoer, G. , V. G. Dethier, and L. M. Schoonhoven (1977): Chemoreceptors in the preoral cavity of the tobacco hornworm, *Manduca sexta*, and their possible function in feeding behavior. *Entomol. exp. appl. 21*, 287-298.

deBoer, G. and F. E. Hanson (1984): Foodplant selection and induction of feeding preference among host and non-host plants in larvae of the tobacco hornworm, *Manduca sexta* . *Entomol. exp. appl. 35,* 177-193.

deBoer, G. and F. E. Hanson (1987a): Differentiation of roles of chemosensory organs in food discrimination among host and non-host plants in larvae of the tobacco hornworm, *Manduca sexta. Physiol. Entomol. 12,* 387-398.

deBoer, G. and F. E. Hanson (1987b): Feeding responses to solanaceous allelochemicals by larvae of the tobacco hornworm, *Manduca sexta. Entomol. exp. appl. 45*, 123-131.

deBoer, G. and F. E. Hanson (1988): The role of leaf lipids in food selection by larvae of the tobacco hornworm, *Manduca sexta. J. Chem. Ecol. 14*, 669-682.

Frazier, J. L. (1986): The perception of plant allelochemicals that inhibit feeding. In: L. B. Brattsten and S. Ahmad (eds), *Molecular Aspects of Insect-Plant Associations*. Plenum. 1-42.

Hanson, F. E. (1970): Sensory responses of phytophagous lepidoptera to chemical and tactile stimuli. In: D. L. Wood, R. M. Silverstein and M. Nakajima (eds), *Control of Insect Behavior by Natural Products*. Academic Press, New York. 81-91.

Jermy, T., F. E. Hanson, and V. G. Dethier (1968): Induction of specific food preference in lepidopterous larvae. *Entomol. exp. appl. 11*, 211-230.

Schoonhoven, L. M. (1969): Gustation and foodplant selection in some lepidopterous larvae. *Entomol. exp. appl. 12*, 555-564.

Schoonhoven, L. M. (1982): Biological aspects of antifeedants. *Entomol. exp. appl. 31*, 57-69.

Waldbauer, G. P. and G. Fraenkel (1961): Feeding on normally rejected plants by maxillectomized larvae of the tobacco hornworm, *Protoparce sexta* (Lepidoptera, Sphingidae). *Ann. Entomol. Soc. Amer. 54,* 477-485.

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THE ROLE OF VARIOUS AMINO ACIDS IN THE PROTEIN COMPENSATORY RESPONSE OF *LOCUSTA MIGRATORIA*

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ABSTRACT

The ability of animals to regulate their intake of specific nutrients is a fundamental issue in the control of feeding. Of particular interest are the means whereby phytophagous insects regulate their protein intake in the face of the highly variable levels of nitrogenous nutrients found in plants. Experiments are described in which a combination of dietary manipulations, injection experiments and electrophysiological investigations of mouthpart taste receptors show that specific nutrient feedbacks from the blood influence qustatory sensitivity to key amino acids, and that such changes are correlated with compensatory feeding behaviour for amino acids.

Key words: *Locusta migratoria*, feeding behaviour, chemoreceptors, feedback, compensation, protein, amino acids.

INTRODUCTION

Compensatory response to dilutions of various dietary nutrients are known in many insects, but the mechanisms are, on the whole, poorly understood (Simpson and Simpson, 1990). The most extensively studied case is that of fifth-instar *Locusta migratoria* nymphs responding to the dilution of the protein component of an artificial diet (Simpson and Abisgold, 1985; Abisgold and Simpson, 1987, 1988).

Detailed observations of the feeding behaviour of locusts eating low (14% dry weight) and high-protein (28% dry weight) diets showed that increased consumption of the former was due to similar sized meals being eaten more frequently (Simpson and Abisgold, 1985). Shorter intermeal intervals on the low-protein diet could arise if insects reduced the duration of the period of quiescence following a meal and/or accepted food more readily upon contact. Subsequent studies implicated differences in blood composition between the low and high-protein fed insects in changes in both of these parameters. The haemolymph of individuals feeding on the high-protein diet had a greater osmolality, and elevated levels of 11 of the 16 amino acids present in the diet. Administering injections designed to raise the osmotic pressure

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and/or the amino acid profile of the blood of low-protein insects up to the levels found in high-protein animals resulted in an increased latency to feed relative to controls receiving an injection isotonic to the haemolymph. Furthermore, the effect was greater with solutions raising osmotic pressure and amino acid levels together, demonstrating both osmotic and amino acid effects (Abisgold and Simpson, 1987). Corresponding electrophysiological studies were performed to assess how the responsiveness of the maxillary palp chemosensilla (important sources of sensory input during food selection) to sugar, amino acid and salt solutions changes under these conditions. While raising haemolymph osmolality by injection had little effect on responsiveness to any of the stimulating solutions, amino acid injections brought about a marked decline in responsiveness specifically to stimulation with an amino acid mix (Abisgold and Simpson, 1988). The implication is that osmotic pressure influences the onset of locomotory activity by acting centrally, while an amino acid feedback modulates peripheral sensitivity to free amino acids in the diet, influencing the animal's readiness to accept food.

The aim of this paper is to evaluate, using this same approach, the role of the various individual amino acids in the compensatory response.

MATERIALS AND METHODS

a) Diet studies. Fifteen diets were made up in the manner described by Simpson and Abisgold (1985). Two of these were the same as the diets used in the earlier studies, i.e. the p-diet, which contained 14% protein, and the P-diet, which contained 28% protein. A third diet, P(aa) was made by enriching the p-diet with 14% dry weight of 16 free amino acids in the proportions in which they occur in hydrolysates of the protein component used in the diets. Of the remaining 12 diets, 11 were variations on the P(aa)-diet in which a single amino acid was omitted from the enriching mix. The amino acids omitted, were those found to differ significantly in concentration in the blood of p- and P-fed locusts (Abisgold and Simpson, 1987). The twelfth diet was P(aa) lacking the remaining 5 amino acids which were found not to differ significantly in concentration in the blood, and also threonine, tyrosine and isuleucine. Diets were nutritionally similar in all respects apart from their protein/amino acid content, their bulks being maintained by the addition of cellulose.

Animals were maintained individually under constant conditions (30°C, 12:12 h light:dark regime) and were allowed to feed on one of the diets for 12h on the third day of the fifth stadium. Diets were dried in an oven to constant weight before and after the experiment and the weight difference was obtained as a value for the amount eaten per locust.

b) Electrophysiological studies. Standard tip recording techniques were used as described by Abisgold and Simpson (1988), modified from Hodgson et al. (1955). After 35 minutes had elapsed since the termination of a low protein meal (ten minutes short of an average intermeal interval on the p-diet), five sensilla on one of the maxillary palps were stimulated with two solutions: a 0.0125M mixture of amino

Table 1 Concentration of each amino acid injected. The amount of each amino acid made up in solution for the injection was calculated as the amount needed to raise the levels found 40 minutes after the previous meal in the haemolymph of low protein-fed insects to those of high protein-fed insects. Solubility problems made it necessary to omit tyrosine from the solution, although dietary omission studies show this to be unimportant.

Amino acid	Amount injected (nmol)
Alanine	283
Methionine	267
Lysine	251
Leucine	217
Glutamine	212
Threonine	134
Isoleucine	133
Valine	111
Phenylalanine	110
Serine	30
(Tyrosine	.....)

acids in 0.05M NaCl, and a 0.0125M solution of lysine in 0.05M NaCl. The composition of the mix was the same as used in Abisgold and Simpson (1988) and included a 41:46:33:47:52:46:37:28 ratio of leucine, glutamine, serine, methionine, phenylalanine, lysine, valine and alanine. One of two injections was administered to each insect, either the complete mix of amino acids needed to raise the amino acid profile up to that of a P-fed insect at that same time since feeding, dissolved in water (see Table 1), or the same injection less lysine (the osmotic pressure of the injection being maintained with xylose). The former solution was the same as used in the earlier studies of Abisgold and Simpson (1987, 1988). After a further ten minutes, the same sensilla were tested again with the same stimulating solutions. For each insect, the mean number of impulses in the first second following stimulation was calculated for each solution before and after injection. Subtracting the mean response before injection from that after, gave the change in impulse number.

The experiment was repeated twice, using alanine and leucine in place of lysine.

RESULTS

a) Diet studies. The mean amount eaten of each deficient diet was compared to the mean amount eaten of the diet containing 50% of the amino acids in free form and 50% in protein form (Pa.a.-diet) and to the low-protein diet (p-diet) (Table 2). P(aa) was eaten in similar quantities to the P-diet, hence addition of the enriching mix of amino acids resulted in reduced consumption relative to the p-diet. There was a gradation of effect of removing a single amino acid from the enriching mix. In order of greatest effect, lysine, alanine, glutamine, methionine, valine, phenylalanine, leucine and serine resulted in significantly more being eaten relative to the P(aa)-diet.

Table 2 Amount (mg) of diet eaten per gram of insect wet weight for locusts fed one of 15 diets. The data are presented as the mean (±S.E.M.) for mg of diet eaten per gram of wet weight of insect during a 12 h period. p-diet had 14% protein; P-diet 28% protein; P(aa)-diet 14% protein and 14% free amino acids; remaining diets were the same as P(aa) but with either a single amino acid omitted from the enriching 14% mix (and thus present only in the protein component of the diet), or, in the case of the last diet listed, less aspartic acid, proline, glycine, arginine, histidine, threonine, isoleucine and tyrosine. The levels of significance refer to differences between the amount eaten for each diet and the mean amount eaten of the P(aa) diet (Fischer's protected L.S.D. method). N=7-13 per diet. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

Amount eaten per weight of insect (mg/g)						Total free amino acids present in the diet (mg/g)
p (low)	P (high)	P (aa)	P (aa) less one amino acid			
154±13 ***	111±9 ns	91±8	-lys	148±7	***	116
			-ala	145±13	***	120
			-glu	137±7	***	100
			-met	135±10	**	122
			-val	131±7	**	118
			-phe	128±9	**	120
			-leu	119±10	*	115
			-ser	118±10	*	122
			-thr	105±13	ns	121
			-tyr	100±7	ns	121
			-iso	97±7	ns	120
			-"unimportant" amino acids	88±7	ns	102

Omission of the first five of these resulted in the diets being eaten as if all amino acids were reduced, that is, in similar quantities to the p-diet. In other words, removal of just one of these amino acids from the enriching mix resulted in the diet being treated as if *all* amino acids were absent from that mix. When those amino acids whose removal did not elicit compensatory feeding (threonine, isoleucine and tyrosine) were removed together with aspartic acid, proline, glycine, arginine and histidine (those amino acids in the diet which did not differ in blood concentration between p- and P-fed insects) there was no difference in amount eaten from P(aa).

There was no correlation between the concentration of an amino acid in the diet and the amount eaten (see Table 2), nor between the rank of the amino acid and the time course of removal from the blood (Abisgold and Simpson, 1987).

b) Electrophysiological studies. In each of the three experiments, the sensilla of control insects receiving an injection of ten amino acids in water, became less responsive to an amino acid stimulating solution containing 8 amino acids, as expected from earlier work (Simpson and Abisgold, 1985) (Fig. 1.).

There was a graded effect of removing a single amino acid from the injection on this feedback; lysine, alanine and leucine assuming the same relative rank as in the diet studies. Even though the other 9 amino acids were raised to P-fed levels, lysine

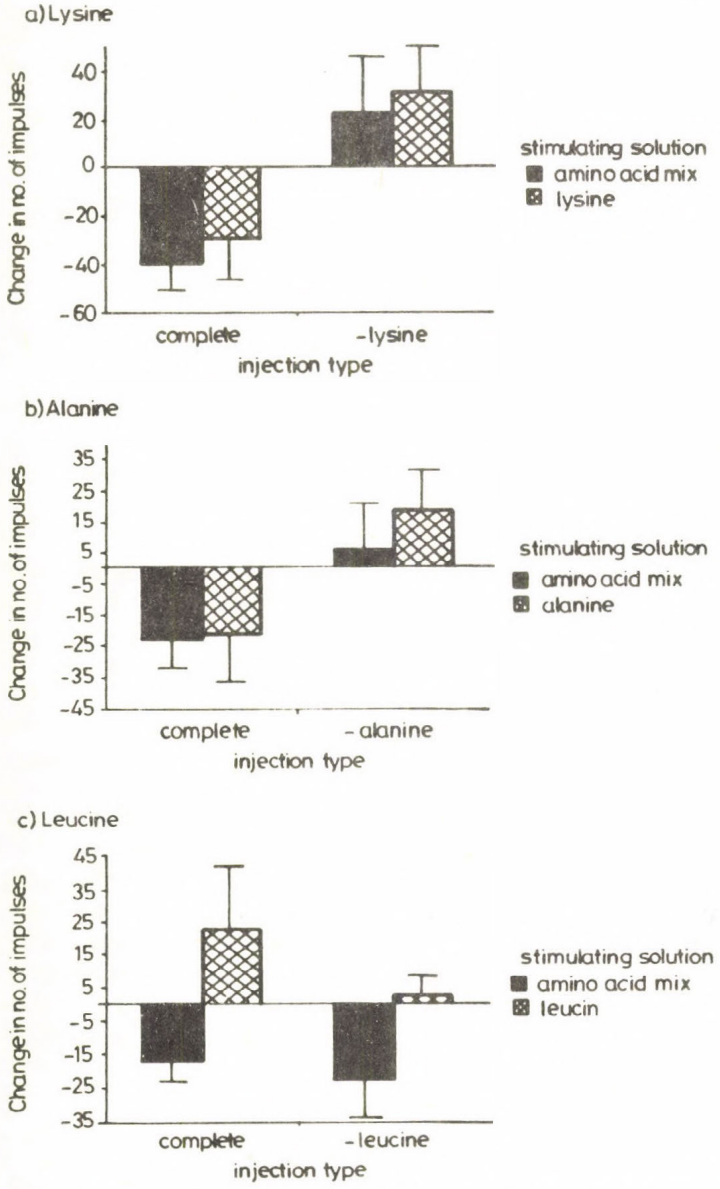

Fig. 1a-c The effect of the nature of the amino acid injection (complete or deficient in one amino acid) on the mean change in the number of impulses (±S.E.M.) elicited from maxillary palp chemosensilla in the first second after stimulation with amino acids. The change in mean response per insect was derived from tests on the same five hairs stimulated before and after injection with two solutions, the data presented being the mean response after injection minus the mean response before injection. The stimulating solutions were a 0.0125M mix of amino acids in 0.05M NaCl, and a 0.0125 M solution of one amino acid in 0.05 M NaCl. N = 8 for each injection. Significance levels are shown in Table 3.

Table 3 Analysis of variance table for experiments omitting single amino acids from the injection mixture. In each case, insects were injected with either the complete amino acid mixture (see text), or the mixture less one amino acid. Responsiveness of the maxillary palp chemosensilla to a solution of 8 amino acids in 0.05M NaCl, and to a solution of the single amino acid in 0.05M NaCl, was tested before and after injection, and the mean increase or decrease in impulse number during the first second of stimulation was calculated for each insect. n=8, $^{*}0.05>p>0.01$, $^{**}0.01>p>0.001$, $^{***}p<0.001$.

Source	d.f.	Amino acid omitted>		
		Lysine	Alanine	Leucine
Injection	1	13.1 **	5.2 *	1.5 ns
Residual 1	14			
Sub-total	15			
Stimulating solution	1	0.2 ns	0.3 ns	11.1 **
Injection x Stim. soln.	1	0.0 ns	0.9 ns	0.3 ns
Residual 2	14			
Total	31			

and alanine had to be present for the feedback on peripheral sensitivity to operate. There was even a suggestion that insects injected with lysine-free and alanine-free solutions exhibited *increased* firing to the amino acid stimulating solution, this was not statistically sifnificant, however. In comparison, removal of leucine did not affect the declining sensitivity to amino acids. The results of the analyses of variance are summarised in Table 3 (see also Figs 1.a-c).

There was variation in the sensitivity of locusts to the single amino acid stimulating solutions. The mean number of impulses (+/-S.E.M.) in the first second of stimulation prior to injection was 61.2 (+/-9.4) for lysine, 37.6 (+/- 9.2) for alanine and 13.3 (+/-2.8) for leucine, while the responses to the mix of 8 amino acids in the three experiments were 58 +/-8.7, 42 +/-6.8 and 40.2 +/-5.7 respectively.

Sensilla of control insects exhibited a post-injection decline in responsiveness to alanine and lysine alone, as well as to the mix of 8 amino acids (Fig. 1.). Omission of one or other of these removed this effect, such that the change in the mean number of impulses did not differ significantly from zero. In contrast, there was no change in responsiveness to stimulation with leucine alone in control or leucine-deficient animals, perhaps reflecting the low pre-injection firing rate of sensilla to this amino acid.

DISCUSSION

The results presented here demonstrate several important features concerning the role of the various amino acids in the compensatory response discovered by Simpson and Abisgold (1985).

Firstly, some of the compensatory responses elicited by deficiencies in individual amino acids are, at least in part, mediated by modulation of peripheral sensitivity.

Locusts responde to dilutions of the concentrations of five of the amino acids as if the entire amino acid complement was reduced, and one might therefore reasonably predict that these five at least, may influence the amino acid feedback on peripheral sensitivity implicated in the compensatory response to the p-diet. The corresponding electrophysiological studies on two of these five, lysine and alanine, support this hypothesis. Removal of either of these from the injection solution offsets the feedback effect. The implication is that deficient animals are more sensitive to free amino acids in the diet than are controls, and thus reject food less often upon contact. This has been confirmed for lysine, since deficient animals have a shorter latency to feeding relative to controls (Simpson, S. J., unpublished data). In addition to a general amino acid feedback, there appear to be individual feedbacks for lysine and alanine, whereby the removal of the amino acid from the injection solution prevents the decline in sensitivity to that amino acid. Experiments are in progress to investigate the specificity of these feedbacks.

Leucine is one of the 8 amino acids whose reduction in the diet elicited a significant compensatory response relative to P(aa)-diet, but the amounts eaten were significantly less than those of locusts fed the p-diet. Electrophysiological tests show no discernible difference between leucine-deficient animals and controls with respect to sentivity to the amino acid mix or leucine alone. In the case of the amino acid mix, the possibility that the highly variable nature of the responses of sensilla masks more subtle effects cannot be excluded, but it is possible that increased consumption is effected by some other mechanism. The low pre-injection firing rate to leucine may explain the absence of an observed feedback effect to stimulation with leucine alone.

The compensatory response is clearly not simply an additive effect of individual amino acids, since the reduction of the dietary concentration of any one amino acid would not be expected to elicit a response as vigorous as a reduction in all amino acids together. This all-or-none phenomenon is borne out in the studies of the feedback on peripheral sensitivity where omission of lysine or alanine from the injection solution completely negates the changes in sensivity apparent with a complete mix.

A gradation of effect of reduction of amino acid levels on the amounts eaten makes it possible to rank the amino acids in order of their importance in stimulating a compensatory response. Electrophysiological data support this ranking for the amino acids studied in two ways: pre-injection sensitivity to the individual amino acids, and the effect of removal of the amino acid from the injection on the feedback, is greatest for lysine and least for leucine.

One of the questions raised by these studies is why one particular suite of amino acids is more important in the regulation of protein intake than any other combination. Various explanations might be proposed. The eight amino acids whose removal elicited a compensatory response relative to P(aa)-diet include five of the "10 rat essentials" (leucine, lysine, methionine, phenylalanine and valine) plus three others (glutamine, serine and alanine). The essentials are those which the rat is unable to synthesize, and there is evidence that for some insects too, many of these must be

obtained from the diet (Dadd, 1985). The synthetic capabilities of *Locusta migratoria*, unfortunately, are unknown.

Some of these amino acids play important roles in the insect's physiology and thus, even if they can be synthesized, may be limiting to growth and development if present in low levels in the diet. It is difficult, however, to make a clear case for the importance of certain amino acids above others on this basis.

Another possibility is that this set of amino acids is, on average, present in low concentrations in host plant and has thus come, during the insect's evolution, to indicate the quality of the food source. Studies of published data on the composition of grasses does not support this, however.

The eight together include representatives from all the major synthetic pathways, as well as being a selection of acidic, basic, neutral, polar and non-polar amino acids. Perhaps this combination of amino acids acts as a sign stimulus, a representative mix, individually important for many different reasons, but which together can be used to ensure a balanced intake of all amino acids.

REFERENCES

Abisgold, J. D. and S. J. Simpson (1987): The physiology of compensation by locusts for changes in dietary protein. *J. exp. Biol. 129,* 329-346.

Abisgold, J. D. and S. J. Simpson (1988): The effect of dietary protein levels and haemolymph composition on the sensitivity of the maxillary palp chemoreceptors of locusts. *J. exp. Biol. 135,* 215-229.

Dadd, R. H. (1985): Nutrition: organisms. In: G. A. Kerkut and L. I. Gilbert (eds), *Comparative Insect Physiology, Biochemistry and Pharmacology*, Pergamon Press, Oxford.

Hodgson, E. S., J. Y. Lettvin and K. D. Roeder (1955): Physiology of a primary chemoreceptor unit. *Science 122,* 417-418.

Simpson, S. J. and J. D. Abisgold (1985): Compensation by locusts for changes in dietary nutrients: behavioural mechanisms. *Physiol. Entomol. 10,* 443-452.

Simpson, S. J. and C. L. Simpson (1990): The mechanisms of nutritional compensation by phytophagous insects. In: E. A. Bernays (ed), *Focus on Insect-Plant Relations.* CRC Press, New York. In press.

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DIETARY SELECTION BEHAVIOUR; COMPARISONS BETWEEN LOCUSTS AND CATERPILLARS

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ABSTRACT

The current nutritional state of larval *Locusta migratoria* and *Spodoptera littoralis* influences their dietary selection. Experiments are described in which insects were conditioned for either 1 meal, 4 h, 8 h or 12 h on diets lacking either protein or digestible carbohydrate. The insects were then provided with the opportunity to choose from diets containing either protein or carbohydrate. The results show that both species exhibit compensatory dietary selection, with *Locusta* responding more rapidly than *Spodoptera*. These behavioural responses are compared to changes in the sensitivity of gustatory receptors.

Key words: Locusta migratoria, Spodoptera littoralis, gustatory receptor, dietary self-selection, protein, carbohydrate, conditioning, feeding, compensation.

Recently there has been a number of investigations on dietary self-selection in phytophagous insects (see reviews by Waldbauer and Friedman, 1988; Simpson and Simpson, 1990). In previous work we have shown that the current nutritional state of larval *Locusta migratoria* (L.) (Simpson, 1982; Abisgold and Simpson, 1987; Simpson and Abisgold, 1985) and *Spodoptera littoralis* (Boisduval) (Simpson et al., 1988, 1989) influences their dietary selection.

MATERIAL AND METHODS

Experiments have been undertaken on insects reared individually from the start of the penultimate larval or nymphal stadium on a nutritionally adequate diet. At a defined stage they were removed from this diet and fed for 1 meal, 4 h, 8 h and 12 h, the conditioning period, on one of two artificial diets that differ in their protein and digestible carbohydrate content. The complete diet, contained 20% protein and 10% digestible carbohydrate. In diet P, the digestible carbohydrate was replaced by cellulose. After the conditioning period, the insects were then provided with an opportunity to choose from diets containing either protein (Diet P) or carbohydrate (Diet C) and thus select the nutrients which were lacking in their previous food. The selection behaviour of the insects was recorded by direct visual observations for 1 h

Table 1 Effects of conditioning diet (4h, 8h and 12h conditioning periods pooled) on the proportion (%) of insects contacting and feeding on the choice diets (Locusts n = 120, *Spodoptera* n = 354). P = protein diet, C = carbohydrate diet.

	Locusta				Spodoptera			
Cond. diet	P		C		P		C	
Choice diet	P	C	P	C	P	C	P	C
1st Contact*	53	37	37	60	34	66	53	47
1st meal@	43	100	91	22	100	100	100	100
Duration (min)#	4.0	7.3	7.9	4.2	11.9	20.7	19.1	26.1
Feeding (%)~	0.9	7.3	5.9	0.9	19.1	33.9	27.4	28.6

* % of total No. of insects conditioned on each conditioning diet that made their initial contact with either the P or C choice diets.
@ % of those contacting the diet that went on to have their first meal on that diet.
# Duration of the 1 st meal.
~ % of 1 h choice period spent feeding on the respective diet.

and then by video for a further 8 h. Full experimental details are given in Simpson et al. (1988, 1989).

RESULTS AND DISCUSSION

When *Locusta* were offered both the P and C choice diets, after only one meal in the conditioning period, the diet they initially contacted was influenced by the nature of the conditioning diet. When conditioned on P, 5 out of 11 locusts contacted the C diet, which contained the nutrients deficient in their previous meal. However, when the conditioning diet was C they were more discriminating, 11 out of 13 contacting the P diet. *Spodoptera* larvae, however, did not show this ability to select for missing nutrients after conditioning for only one meal: 10 out of 20 larvae conditioned on P made their initial contact with C and 9 out of 20 conditioned on C contacted P.

These initial contacts did not always lead to sustained feeding on the choice diets. Of the locusts conditioned on the P diet, only 6% took meals of greater than 30 seconds duration on the P diet compared to 46% on the C diet. Of those conditioned on the C diet, 24% took such meals on the C diet compared to 51% on the P diet. That is to say, when the duration of the first period of feeding is considered, it is clear that *Locusta* compensated for the nutrients missing in the conditioning diet, even after a conditioning period of only one meal.

By contrast, all *Spodoptera* larvae took meals of greater than 30 seconds duration on the choice diets which they first contacted. Thus, the conditioning diets did not significantly influence the acceptance behaviour of the larvae on either of the choice diets.

When the conditioning period is longer than one meal different selection behaviour occurs in the two species (Table 1). In general, *Locusta* made that first contact with the diet on which they had been conditioned. In comparison, *Spodoptera* conditioned on P initially contacted the C choice diet more frequently than the P choice diet, but larvae conditioned on the C diet still contacted equally the P and C choice diets. However, when the diet initially contacted by *Locusta* was the one appropriate for compensation, they fed on it, whereas *Spodoptera* larvae always fed on whichever diet they first contacted (Table 1).

The durations of the first meals taken by locusts also indicate their ability to compensate (Table 1). The meal duration on the choice diet containing the deficient nutrient was significantly greater than on the choice diet corresponding to the diet on which conditioning occurred. The same phenomenon was shown by *Spodoptera* larvae conditioned on the P diet but not by those conditioned on the C diet (Table 1). The *Spodoptera* larvae always took longer first meals on the C choice diet.

A similar pattern of behaviour on the choice diets is seen in the proportion of time in the first hour spent feeding (Table 1), except that in this case *Spodoptera* larvae conditioned on C spent equal amounts of time feeding on the P and C choice diets.

It is evident from the foregoing that *Spodoptera* larvae do not compensate so readily as the locusts during the initial hour of the choice period. However, previous

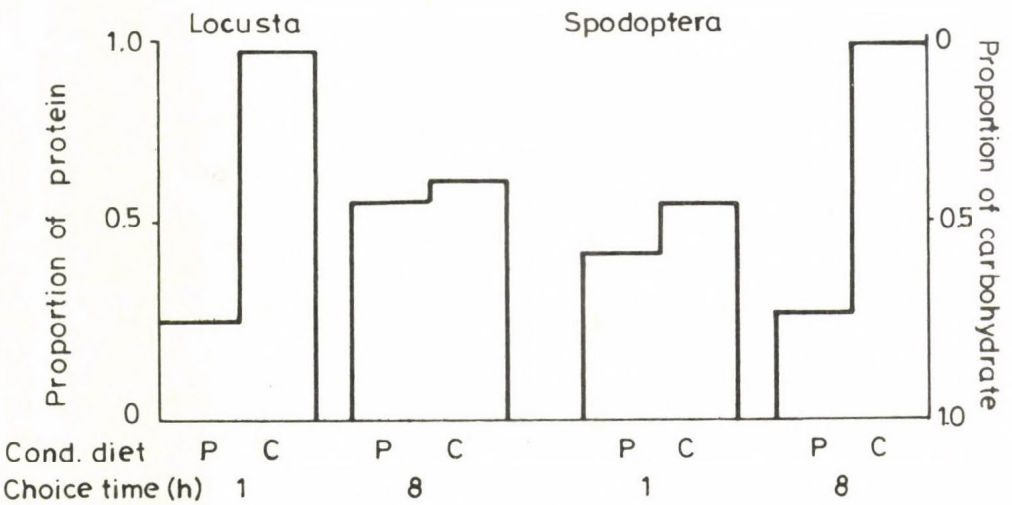

Fig. 1 Proportions of protein and digestible carbohydrate consumed by nymphs and larvae eating the choice diets (P and C) during the first hour and subsequent 8 hours choice period for insects conditioned for 8 hours on the P or C diets. (*Locusta* n = 20; *Spodoptera* n = 60)

work (Simpson et al., 1988) in which the amounts of food eaten were considered, has shown that *Spodoptera* larvae do compensate when they have been conditioned for longer than 4 hours, provided the choice period extends for another 8 hours.

The diets used in our experiments were chosen to reflect the amounts of protein and carbohydrate (29% and 10%, respectively) found in seedling wheat. When this is taken into account, and the actual amounts of protein and carbohydrate consumed in the choice period are compared for the two species, over the one hour and eight hour choice periods, the differences in speed with which compensation is achieved becomes apparent (Fig. 1). With both conditioning diets, most of the compensation by locusts is achieved during the first hour of choice and selection over the eight hour period shows little difference between amounts of P and C choice diets eaten. With *Spodoptera* larvae the reverse applies: little difference is apparent during the first hour, but over the full eight hour period compensatory feeding is very marked (Fig. 1).

The compensatory feeding behaviour seen in these experiments in response to dietary deficiencies could be associated with changes in sensitivity of peripheral taste receptors, altering the way in which the diets are perceived by the insects. Alternatively, the behaviour could be determined by changes having their effect in the central nervous system, or by a combination of these two principles.

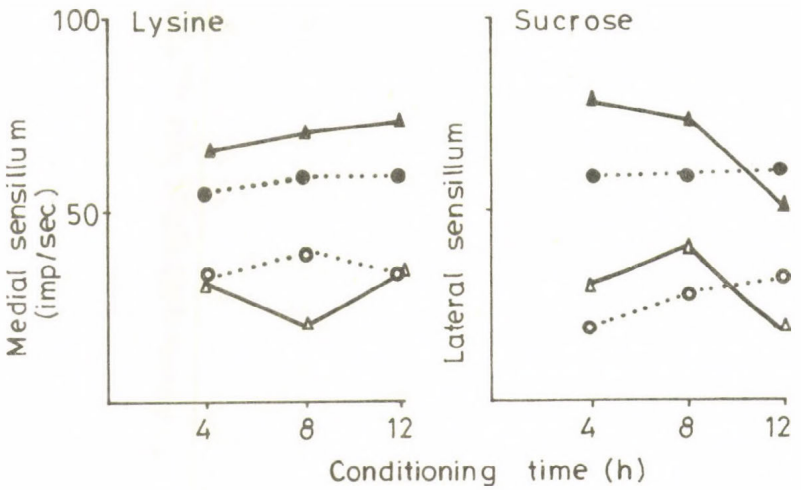

Fig. 2 Effects that conditioning for different periods of time, on the P and C diets, have on the electrophysiological response of the maxillary styloconic sensilla of *Spodoptera littoralis* larvae to stimulation with lysine (0.01 M) and sucrose (0.05 M). (10 replicates per point, conditioned on P = —, C = ...; response immediately after larvae removed from conditioning diet = Δ O, response of larvae about to start feeding on a C diet ▲ ●)

Investigations of the taste receptors on the maxillary palps of *Locusta* nymphs (Abisgold and Simpson, 1988; Simpson et al., this volume) have shown that the sensitivity of the receptors to amino acids is decreased when the nymphs have a high blood amino acid level, as occurs after a meal. Similarly, when the maxillary styloconic sensilla of *Spodoptera* larvae are recorded from soon after a meal, such as the conditioning meals, the sensitivity of the sensilla is lower (Fig. 2) than that found immediately before the next meal is taken (Fig. 2). There is a greater difference in response between the post conditioning and pre-meal recordings with P as the conditioning diet than there is with C. This could result in longer intervals between feeds in the P conditioned insects which is in accord with the findings of Simpson et al. (1988) who found that meal size was determined largely by the amount of C diet eaten whereas intermeal intervals were influenced by the intake of P diet. The effect of the duration of the conditioning time is, generally, to increase the response of neurones in the lateral sensillum to sucrose and in the medial sensillum to lysine, regardless of the nature of the conditioning diet.

The one apparent anomaly is the effect that 12 hours conditioning on the P diet has on the response of the lateral sensillum to sucrose (Fig. 2). This effect was

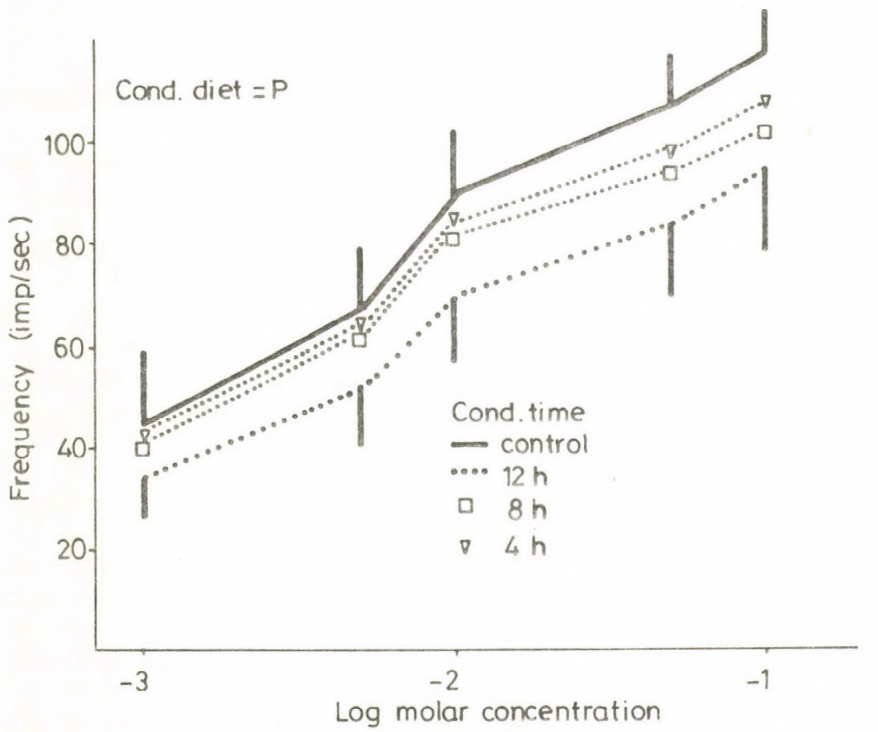

Fig. 3 Responses of lateral styloconic sensilla of larvae of *Spodoptera littoralis* to stimulation with five concentrations of sucrose applied after different periods of conditioning on diet P. (n = 40; conditioning period 0 (control) = —, 4h = ▽, 8h = □, 12h = •••).

investigated further, stimulating the lateral sensilla of larvae conditioned on the P diet with a range of concentrations of sucrose (Fig. 3). The post conditioning responses shown here reveal a trend to decreasing firing rate with increasing conditioning time. The response after 12 hours conditioning is significantly different from the control level at all concentrations tested, except at the lowest (χ^2; $p>0.01$).

In conclusion, we have shown that both *Locusta* and *Spodoptera* have the ability to compenstate by differential feeding for deficiencies of protein or digestible carbohydrate in their diets. Compensation occurs much more rapidly in *Locusta*, being brought into play after only one deficient meal and being largely complete within 1 hour of exposure to the compensatory diets. Under the same conditions, *Spodoptera* respond after 8 hours on a deficient diet and show substantial compensation only during the 8 hour exposure to the compensatory diets. The activity of the peripheral taste receptors of *Spodoptera* show changes in sensitivity after conditioning, especially in the response to sucrose for larvae conditioned on the P diet for 12 hours. Evidently, decreased firing rate with deprivation is associated with increased intake of the C choice diet.

REFERENCES

Abisgold, J. D. and S. J. Simpson (1987): The physiology of compensation by locusts for changes in dietary protein. *J. Exp. Biol. 129,* 329-346.

Abisgold, J. D. and S. J. Simpson (1988): The effect of dietary protein levels and haemolymph composition on the sensitivity of the maxillary palp chemoreceptors of locusts. *J. Exp. Biol. 135,* 215-229.

Simpson, S. J. (1982): Changes in the efficiency of utilization of food throughout the fifth instar of *Locusta migratoria* nymphs. *Entomol. exp. appl. 31,* 265-275.

Simpson, S. J. and J. D. Abisgold (1985): Compensation by locusts for changes in dietary nutrients: behavioural mechanisms. *Physiol. Entomol. 10,* 443-452.

Simpson, S. J. and C. L. Simpson (1990): Mechanisms of nutritional compensation by phytophagous insects. In: E. A. Bernays (ed), *Insect-Plant Interactions, Vol. 2,* CRC Press, (in press).

Simpson, S. J., M. S. J. Simmonds and W. M. Blaney (1988): A comparison of dietary selection behaviour in larval *Locusta migratoria* and *Spodoptera littoralis*. *Physiol. Entomol. 13,* 225-238.

Simpson, S. J., M. S. J. Simmonds, W. M. Blaney and J. P. Jones (1989): Compensatory dietary selection occurs in larval *Locusta migratoria* but not *Spodoptera littoralis* after a single meal during *ad libitum* feeding. *Physiol. Entomol.* (in press).

Waldbauer, G. P. and S. Friedman (1988): Dietary self-selection by insects. In: F. Sehnal, A. Zabza and D. L. Denlinger (eds), *Endocrinological Frontiers in Physiological Insect Ecology.* Wroclaw Technical University Press, Wroclaw. 403-422.

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PROCESSING OF PYRROLIZIDINE ALKALOIDS AND CARDENOLIDES IN THREE MOTHS, *SYNTOMIS MOGADORENSIS, SYNTOMEIDA EPILAIS* AND *CREATONOTOS TRANSIENS*

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ABSTRACT

The processing of plant-derived allelochemicals, especially pyrrolizidine alkaloids and cardenolides, was studied in three moths. *Syntomis mogadorensis* is polyphagous and prefers less noxious food plants in general. However, before starving also plants containing alkaloids, terpenes or glycosides are accepted. Non-resorption of these metabolites and additionally detoxification seem to be the main strategies to overcome the toxicity of the secondary metabolites in this situation. *Syntomeida epilais* is a specialist with a small range of food plants, like *Nerium oleander*, producing cardiac glycosides. Larvae selectively sequester cardenolides and probably exploit them for their own defence. *Creatonotos transiens* must be grouped in between these classes: Larvae are polyphagous and have similar strategies for dealing with noxious plants and compounds as *S. mogadorensis*. At least under laboratory conditions it is a specialist for pyrrolizidine alkaloids: PA are selectively taken up and stored in the integument. In females, PA are transferred to the eggs to a high degree. In males, PA serve as a morphogen for the development of an abdominal corema and as precursor for the pheromone hydroxydanaidal.

Key words: secondary metabolites, resorption, detoxification, pheromone, pyrrolizidine alkaloid, hydroxydanaidal, Arctiidae, Ctenuchidae.

CHEMICAL DEFENSE OF PLANTS

A characteristic feature of plants is the production of secondary metabolites which are now understood as allelochemicals, i.e. protecting agents. Several thousands of them have been discovered so far, with over 4500 terpenes, 2400 phenolics, 7000 alkaloids, 400 non-protein amino acids, 50 cyanogenic glycosides and 100 glucosinolates. Due to the high sophistication of modern chromatography and spectroscopy many more structures can be expected in the future, the more so as only 10-15% of all plants have been analyzed by now. It has been become clear during the last 3 decades that secondary metabolites function in the plant as the main line of defence against enemies, i.e. microorganisms and herbivores. In addition, secondary metabolites can be attractants for pollen- and seed dispersing animals or may even serve in the primary physiology of certain plants (Harborne, 1982; Swain, 1977; Levin, 1976; Rosenthal and Janzen, 1979; Schlee, 1986; Wink, 1988).

Table 1 Palatability of food plants for *Syntomis mogadorensis* and *Creatonotos transiens*. Details in Wink and Schneider (1990).

	S. mogadorensis	*C. transiens*
A. Choice tests		
Number of plant species	44	74
Proportion of leaves eaten		
0 %	25	36
1-20 %	15	12
21-80 %	1	14
80-100 %	3	12
B. No-Choice		
Number of plant species	130	91
Proportion of leaves eaten		
0 %	32	17
1-20 %	46	10
21-80 %	27	3
81-100 %	24	61

The formation and accumulation of these compounds by plants is a highly complex phenomenon from biosynthesis, intra- and intercellular transport, storage, to degradation. These processes are compartmentalized and regulated at the intra- and intercellular and developmental level and are coordinated and adapted in such a way that these compounds can fulfil their allelochemical functions. These aspects have been studied thoroughly in a limited number of plant species only (for review Wink, 1987a,b). However, such facts are essential, if we want to understand and interpret the interactions between plants and animals and the adaptations of herbivores towards plants as a food source.

ADAPTATIONS OF HERBIVORES TOWARDS PLANT ALLELOCHEMICALS

During the long history of evolution, herbivores developed several strategies to avoid, tolerate, overcome or even utilize the chemical defence of plants. Since many herbivore species have evolved a unique and special way to deal with their host plants, an overall generalization cannot be given and is dangerous in particular, because our knowledge of the underlying intricate biochemical and physiological interactions is often very limited.

We have studied larvae and later developmental stages of three Lepidoptera (two brilliantly coloured, lazily flying, diurnal ctenuchids, *Syntomeida epilais*, and *Syntomis mogadorensis*, and a nocturnal arctiid, *Creatonotos transiens*) and have analyzed,

1. the influence of plant allelochemicals on larval food choice,
2. the fate of cardiac glycosides (CG) and alkaloids in these insects, and

3. the biochemical adaptions in the two specialists, which store and exploit some of the dietary chemicals.

Syntomis mogadorensis. Dandelion, *Taraxacum officinale* is a favourite food plant of *S. mogadorensis* larvae which we either reared for many generations on this plant, or on a simple, defined diet (Bergomaz and Boppré, 1986). In palatability tests the hairy, cryptic larvae were offered 44 plant species, which contained a wide variety of allelochemicals, such as alkaloids, glycosides and terpenes in addition to *Taraxacum* (Table 1). 40 of these species were not or hardly eaten under choice conditions. Under no-choice conditions, i.e. before starving, 18% of 130 species were almost completely and another 21% partly accepted (Wink and Schneider, 1990).

Since the adult moths are colourful, aposematic insects, we were interested whether they sequestered plant allelochemicals and exploit them as acquired defence compounds. However, of more than 10 allelochemicals studied, including alkaloids and cardiac glycosides, none was found to be stored in the body, but the faeces contained the allelochemicals in question (Wink and Schneider, 1990). A likely feeding strategy of *S. mogadorensis* is to refuse noxious plants, and if this cannot be helped, not to resorb the toxins. A fast midgut passage and a high selectivity of the dietary uptake systems for the essential nutrients (amino acids, lipids, sugars) would be plausible mechanisms to realize such a strategy. We have not yet analyzed whether intestinal or microsomal detoxification degrade those unwanted xenobiotics which enter the cells by simple diffusion.

Syntomeida epilais. Moths of *S. mogadorensis* and of *Syntomeida epilais* are similar in appearance. Whereas we have not found any chemical defence in *S. mogadorensis* yet, it was discovered already by Rothschild et al., (1973) that the warningly coloured larvae and moths of *S. epilais* sequestered cardiac glycosides. *S. epilais* larvae are oligophagous and only feed on *Echites* and *Nerium oleander* (Apocynaceae) which produce cardiac glycosides. Yet CG are not the "feeding key", since other plants with CG were not accepted (Wink and Schneider, 1990). Dietary CG were partly recovered from the larvae (5.4 mg/g FW) but also from the faeces. Our recent, but preliminary experiments indicate that the resorption of CG by the intestinal epithelia is not due to simple diffusion, but to a selective transport system (unpublished data).

Creatonotos transiens. The South-East Asian arctiid, *Creatonotos transiens*, has aposematic imagines. Larvae are cryptic and polyphagous in nature and can be kept in the laboratory on a defined diet (Bergomaz and Boppré, 1986; Boppré and Schneider, 1989). In palatability experiments (see *S. mogadorensis*) larvae accepted 26 of 74 plant species. Under "no-choice" conditions the proportion of acceptable species raised to 67% (Table 1). The fate of alkaloids was similar as in *S. mogadorensis* with one remarkable exception (Wink and Schneider, 1990): Pyrrolizidine alkaloids (PA) were selectively taken up. Only if the PA dosage exceeded 2 mg/meal, part of the PA could be recovered from the faeces (Wink and Schneider, 1990). In our experiments with larvae that had obtained 3 mg PA in L7, 13% PA was recorded from the faeces (Fig. 1). We have shown recently that the resorption of PA is achieved through a specific transport system (Wink and Schneider, 1988), which is absent from *S. mogadorensis*, a species that does not sequester PA. After the PA molecules

are resorbed, at least three biochemical conversions take place: 1. a 95% oxidation of free PA to its N-oxide (Egelhaaf et al., 1989; for other arctiids see Mattocks, 1971; Culvenor and Edgar, 1972). 2. a stereochemical inversion of the hydroxylgroup at C7 of 7S-heliotrine to the 7R-form (Wink et al., 1988; Bell et al., 1984; Bell and Meinwald, 1986). 3. The recovery rates of dietary PA were between 50% and 90% in a Bali and Philippine strain, respectively. We attribute the respective "loss" of PA to degradation ("detoxification").

We have analyzed the distribution of the resorbed PA in larvae and later developmental stages of *C. transiens* and found, that PA are predominantly stored in the integument (Egelhaaf et al., 1989). In the imagines, a major redistribution takes place, in that in females, up to 80% of PA is transferred to the eggs (Fig. 1), a

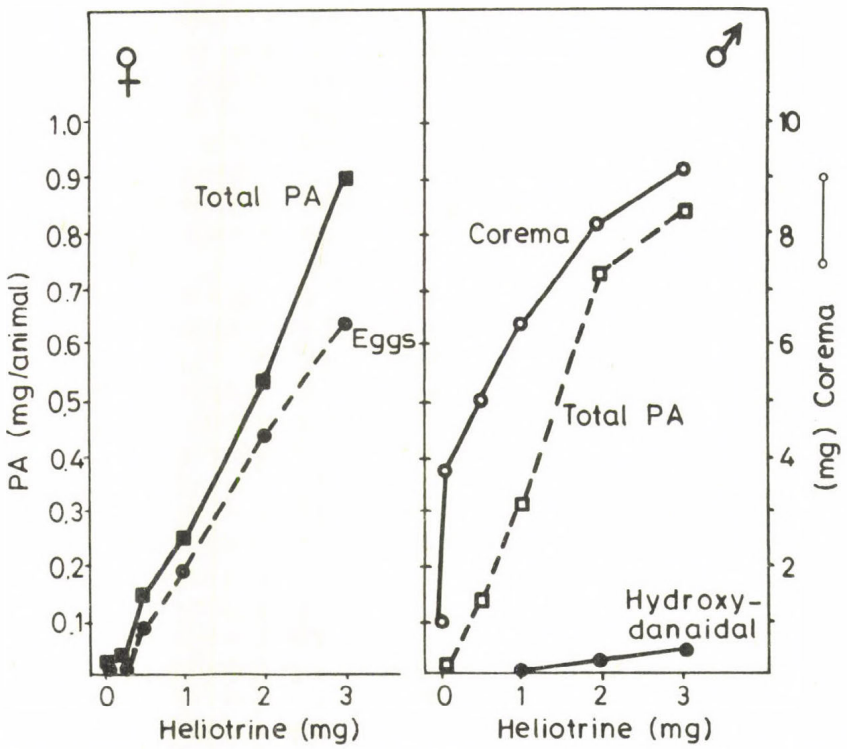

Fig. 1 Fate of dietary PA in *Creatonotos transiens*. L7 larvae were fed with appropriate amounts of heliotrine. Males (n=3) were analyzed 1 day and females (n=3) 7 days after eclosion. Coremata were inflated, and cut off the body. After determination of fresh weight (given in mg on the right ordinate) and size, coremata were taken up in 200 μl ethylacetate. 1 μl aliquots were analyzed by capillary GLC (0.3 mm x 30 m; DB-1) with a Varian 3300 GLC, equipped with a nitrogen specific detector. PA were extracted from animals and analyzed by capillary GLC as described in Wink and Schneider (1988). All values are means of 3 animals. In females, PA recovered from body and all eggs are given as "Total PA". In males, the hydroxydanaidal contents are not included in the "Total PA" values.

Table 2 Hydroxydanaidal and PA contents of *Creatonotos transiens* in relation to corema development. Comparison between laboratory reared animals (PA containing and PA-free diet versus animals field-caught in the Philippines and Bali). Extraction and PA analysis as in Fig. 1. T: corema type (+++=fully developed corema (s. Fig. 1 for 3 mg dietary PA.) L: Corema size: length of outer branch of the inflated corema in mm.

Animal	Origin/Treatment	Corema development		Hydroxydanaidal content	PA-content body
		T	L	corema µg/corema	µg/animal
1	field	+++	n.d.	34	<2
2	field	+++	n.d.	102	<2
3	field	+++	n.d.	57	<2
4	field	+++	n.d.	138	<2
5	field	+++	n.d.	72	<2
6	field	+++	n.d.	187	<2
7	field	+++	n.d.	0	<2
8	field	+++	n.d.	79	<2
9	field	+++	39	261	<2
10	field	+++	28	20	<2
11	field	+++	28	205	<2
12-42	field	n.d.	n.d.	n.d.	<2
43	lab/+PA$^{\bullet}$	+++	33	11	913
44	lab/+PA$^{\bullet}$	+++	35	97	809
45	lab/+PA$^{\bullet}$	+++	35	52	1189
46	lab/+PA$^{\bullet}$	+++	42	49	615
47	lab/+PA$^{\bullet}$	+++	35	47	769
48	lab/+PA$^{\bullet}$	+++	36	44	1308
49	lab/+PA$^{\bullet}$	+++	36	55	1388
50	lab/+PA$^{\bullet}$	+++	37	104	1009
51	lab/+PA$^{\bullet\bullet}$	+++	42	239	446
52	lab/+PA$^{\bullet\bullet}$	+++	40	203	181
53	lab/+PA$^{\bullet\bullet}$	+++	43	307	88
54	lab/+PA$^{\bullet\bullet}$	+++	34	366	271
55	lab/+PA$^{\bullet\bullet}$	+++	36	271	262
56	lab/+PA$^{\bullet\bullet}$	+++	40	228	473
57	lab/+PA$^{\bullet\bullet\bullet}$	+++	46	141	356
58	lab/+PA$^{\bullet\bullet\bullet}$	+++	42	188	407
59	lab/+PA$^{\bullet\bullet\bullet}$	+++	39	179	326
60	lab/+PA$^{\bullet\bullet\bullet}$	+++	43	96	432
61	lab/+PA$^{\bullet\bullet\bullet}$	+++	38	168	201

•= 3 mg heliotrine in diet, applied in L7

••= larvae had obtained PA-containing *Senecio vulgaris* (L1 to L7) or *S. vernalis* (•••) in L7.

1-27: Phillipines[1], 28-42: Bali

[1] The Philipine strain of *C. transiens* has been referred to as *Creatonotos wilemani* (Inoue, 1988).

phenomenon which has also been recorded for other arctiids (Benn et al., 1979; Dussourd et al., 1988). In males, PA display an extraordinary feature: If PA are present in the diet, large abdominal scent organs, ("coremata") are formed (Schneider et al., 1982; Boppré and Schneider, 1985) (Fig. 1). The ability to induce corema development requires the presence of ecdysone and terminates in the prepupal stage (Schmitz et al., 1989). In males, PA appear to have an additional function as a precursor for the pheromone 7R-hydroxydanaidal (Boppré and Schneider, 1985; Wunderer et al., 1986; Bell and Meinwald, 1986; Schneider, 1987) (Fig. 1), a feature also assumed for hydroxydanaidal in other arctiids and danaines (for the literature see Bell and Meinwald, 1986; Boppré, 1986; Conner et al., 1981; Culvenor and Edgar, 1972; Edgar and Culvenor, 1974; Krasnoff and Dussourd, 1989).

Earlier electroantennogram recordings from danaines and arctiids indicated the capacity of the olfactory system in both sexes to sense the different dihydropyrrolizidine pheromones (Schneider and Seibt, 1969; Schneider et al., 1975; Boppré, 1986). In the meantime, receptor cells specific for both hydroxydanaidal stereoisomers have been found in *Creatonotos* (Wunderer et al., 1986) and in *Rhodogastria* (Bogner and Boppré, 1989).

The correlation between dietary PA storage, large coremata and high hydroxydanaidal values can be reproducibly established in the laboratory (Fig. 1, Table 2). However, when we analyzed males which were collected in Bali or in the Philippines from field populations, we found large, fully developed corema with a high amount of hydroxydanaidal but only traces of PA (Table 2). Most of the males studied were dead when they finally reached our laboratory. The possibility of a substantial PA-breakdown in dead animals can be ruled out by the fact that moths which arrived in Germany alive, were also nearly PA-free and we know that the PA-content of imagines is rather stable throughout their life span.

To explain the obvious discrepancy between laboratory and field data, we need first to note that possibly all the field-caught specimens had lived on rice or other Gramineae (which are PA-free) and not on a true PA plant (Boppré and Schneider, 1990). This might explain the lack of PA-storage in the moths. But how to explain the large coremata with their high hydroxydanaidal contents? The most rigorous speculation would be that we are still overlooking one (or *the*) critical factor (with or without PA) in the chain of processes of the corema induction and pheromone production. Could it be that *Creatonotos* has in principle the endogenous capacity to synthesize hydroxydanaidal after the respective biosynthetic enzymes have been induced by an unknown dietary factor (or a PA, if available)? Thus PA storage, corema induction and pheromone biosynthesis may be not as tightly correlated in the wild as our laboratory experiments suggest. We wonder, whether PA-uptake, storage and pheromone production is better correlated in any other lepidopteran (Boppré, 1986; Conner et al., 1981; Krasnoff et al., 1987; Krasnoff and Dussourd, 1989; Rothschild et al., 1979).

The impressive corema with their pheromones suggest that this feature should be important for courtship and reproductive success. At least in the laboratory we could again not see an effect on reproductivity whether we had males with large or minute

corema and respective high or low hydroxydanaidal contents (Boppré and Schneider, 1990; Wunderer et al., 1986). A detailed study concerning fitness and reproductive success under field conditions is certainly needed to answer this question.

The storage of PA in the integument and their transfer to the eggs in the female suggest that PA function as acquired defence compounds, important for the survival of this moth. Laboratory experiments show that PA-loaded animals, such as ithomiine butterflies or *C. transiens* are indeed protected against predators (Brown, 1984; Boppré, 1986). The more, it is surprising that in no case we have come across a *C. transiens* moth in the field with a high PA-content (Table 2). It might be argued that the capability for PA processing was acquired by *Creatonotos* long ago in its evolutionary history but that it does not exploit it in the populations studied by us. Maybe other natural products are sequestered or synthesized in these populations, which have escaped our chemical investigations.

C. transiens can be considered as a generalist, but also a PA specialist, which has evolved very intricated biochemical and physiological adaptions for storage, processing and exploitation of dietary PA which can be used if the opportunity is provided.

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REFERENCES

Bell, T. W., M. Boppré, D. Schneider and J. Meinwald (1984): Stereochemical course of pheromone biosynthesis in an arctiid moth, *Creatonotos transiens*. *Experientia 40*, 713-714.

Bell, T. W. and J. Meinwald (1986): Pheromones of two arctiid moths (*Creatonotos transiens* and *C. gangis*) chiral components from both sexes and achiral female components. *J. Chem. Ecol. 12*, 385-409.

Benn,M., J. DeGrave, C. Gnanasunderam and R. Hutchins (1979): Host-plant pyrrolizidine alkaloids in *Nyctemera annulata* Boisduval: Their persistence through the life cycle and transfer to a parasite. *Experientia 35*, 731-732.

Bergomaz, R. and M. Boppré (1986): A simple instant diet for rearing Arctiidae and other moths. *J. Lep. Soc. 40*, 131-137.

Bogner, F. and M. Boppré (1989): Single cell recordings reveal hydroxydanaidal as the volatile compound attracting insects to pyrrolizidine alkaloids. *Entomol. exp. appl. 50*, 171-184.

Boppré, M. (1986): Insects pharmacophagously utilizing defensive plant chemicals. *Naturwiss. 73*, 17-26.

Boppré, M. and D. Schneider (1985): Pyrrolizidine alkaloids quantitatively regulate both scent organ morphogenesis and pheromone biosynthesis in male *Creatonotos* moths. *J. Comp. Physiol. 157*, 569-577.

Boppré, M. and D. Schneider (1989): The biology of *Creatonotos* (Lepidoptera: Arctiidae) with special reference to the androconial system. *Zool. J. Linn. Soc.* (in press).

Brown, K. S. (1984): Adult-obtained pyrrolizidine alkaloids defend ithomiine butterflies against a spider predator. *Nature 309*, 707-709.

Conner, W. E., T. Eisner, R. K. VanderMeer, A. Guerrero and J. Meinwald (1981): Precopulatory sexual interaction in the arctiid moth (*Utetheisa ornatrix*): Role of a pheromone derived from dietary alkaloids. *Behav. Ecol. Sociobiol. 9*, 227-235.

Culvenor, C. C. J. and J. A. Edgar (1972): Dihydropyrrolizidine secretions associated with corema of *Utetheisa* moths (Family Arctiidae). *Experientia 28*, 627-628.

Dussourd, D. E., K. Ubik, C. Harvis, J. Resch and J. Meinwald (1988): Biparental defensive endowment of eggs with acquired plant alkaloid in the moth *Utetheisa ornatrix. Proc. Natl. Acad. Sci. 85*, 5992-5996.

Edgar, J. A. and C. C. J. Culvenor (1974): Pyrrolizidine ester alkaloid in danaid butterflies. *Nature 248*, 614-616.

Egelhaaf, A., K. Cölln, B. Schmitz, M. Buck, M. Wink and D. Schneider (1989): Distribution of pyrrolizidine alkaloids in the arctiid moth, *Creatonotos transiens. Z. Naturforsch.* (in prep.).

Harborne, J. B. (1982): *Introduction to Ecological Biochemistry.* Academic Press, London.

Inoue, H. (1988): Three new species and some synonymic notes on the Arctiidae from Japan, Taiwan and the Philippines. *Tyo to Ga 39*, 99-118.

Krasnoff, S. B., L. B. Bjostad and W. L.Roelofs (1987): Quantitative and qualitatative variation in male pheromones of *Phragmatobia fuliginosa* and *Pyrrharctia isabella* (Lepidoptera: Arctiidae). *J. Chem. Ecol. 13*, 807-822.

Krasnoff, S. B. and D. E. Dussourd (1989): Dihydropyrrolizidine attractants for arctiid moths that visit plants containing pyrrolizidine alkaloids. *J. Chem. Ecol. 15*, 47-60.

Levin, D. A. (1976): The chemical defences of plants to pathogens and herbivores. *Ann. Rev. Ecol. Syst. 7*, 121-159.

Mattocks, A. R. (1971): Occurrence and analysis of pyrrolizidine alkaloid N-oxides. *Xenobiotica 1*, 451-453.

Rosenthal, G. A. and D. Janzen (1979): *Herbivores: Their Interaction with Plant Secondary Metabolites.* Academic Press, London.

Rothschild, M., J. v. Euw and T. Reichstein (1973): Cardiac glycosides in the polka-dot moth *Syntomeida epilais* with some observations on the toxic qualities of *Amata phegea. Proc. R. Soc. Lond. B 183*, 227-247.

Rothschild, M., R. T. Aplin, P. A. Cockrum, J. A. Edgar, P. Fairweather and R. Lees (1979): Pyrrolizidine alkaloids in arctiid moths (Lep.) with a discussion on host plant relationships and the role of these secondary plant substances in the Arctiidae. *Biol. J. Linn. Soc. 12*, 305-326.

Schlee, D. (1986): *Ökologische Biochemie.* Springer, Berlin.

Schmitz, B., M. Buck, A. Egelhaaf and D. Schneider (1989): Ecdysone and a dietary alkaloid interact in the development of the pheromone gland of a male moth. *Roux's Arch. Dev. Biol.* (in press).

Schneider, D. (1987): The strange fate of pyrrolizidine alkaloids. In: R. F. Chapman, E. A. Bernays and J. G. Stoffolano. (eds.) *Perspectives in Chemoreception and Behavior.* Springer, New York. 123-142.

Schneider, D., M. Boppré, H. Schneider, W. R. Thompson, C. J. Boriack, R. L. Petty and J. Meinwald (1975): A pheromone precursor and its uptake in male *Danaus* butterflies. *J. Comp. Physiol. 97*, 245-256.

Schneider, D., M. Boppré, J. Zweig, S. B. Horsley, T. W. Bell, J. Meinwald, K. Hansen and E. W. Diehl (1982): Scent organ development in *Creatonotos* moths: regulation by pyrrolizidine alkaloids. *Science 215*, 1264-1265.

Schneider, D. and U. Seibt (1969): Sex pheromone of the queen butterfly: Electroantennogram responses. *Science 164*, 1173-1174.

Swain, T. (1977): Secondary compounds as protective agents. *Ann. Rev. Plant Physiol. 28*, 479-501.

Wink, M. (1987a): Physiology of the accumulation of secondary metabolites with special references to alkaloids. In: F. Constabel (ed), *Cell Culture and Somatic Cell Genetics of Plants*. Academic Press, London. 17-42.

Wink, M. (1987b): Chemical ecology of quinolizidine alkaloids. In: G. R. Waller (ed), *Allelochemicals: Role in Agriculture, Forestry and Ecology*. Am. Chem. Soc., Washington. 330, 524-533.

Wink, M. (1988): Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. *Theor. Appl. Genet. 75*, 225-233.

Wink, M. and D. Schneider (1988): Carrier-mediated uptake of pyrrolizidine alkaloids in larvae of the aposematic and alkaloid-exploiting moth, *Creatonotos*. *Naturwiss.* 75, 524-525.

Wink, M. and D. Schneider (1990): Fate of plant derived secondary metabolites in three moth species *(Syntomis mogadorensis*, *Syntomeida epilais*, and *Creatonotos transiens*). *J. Comp. Physiol.B* (in prep.)

Wink, M., D. Schneider and L. Witte (1988): Biosynthesis of pyrrolizidine alkaloid-derived pheromones of the arctiid moth, *Creatonotos transiens*: Stereochemical conversion of heliotrine. *Z. Naturforsch. 43c*, 737-741.

Wunderer, H., K. Hansen, T. W. Bell, D. Schneider and J. Meinwald (1986): Sex pheromones of two Asian moths, *Creatonotos* (Lepidoptera: Arctiidae): behaviour, morphology, chemistry and electrophysiology. *Exp. Biol. 46*, 11-27.

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INSECT RESPONSES TO PRO-OXIDANT PLANT ALLELOCHEMICALS

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ABSTRACT

The phytophagous insects are subject to both endogenous and exogenous sources of oxidative stress from toxic oxygen radicals, H_2O_2 and lipid peroxides (LOOH). Therefore, antioxidant enzymatic defense of insects for the regulation of oxygen toxicity was investigated. The activities were recorded for the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPOX), glutathione transferase (GST), and glutathione reductase (GR) in three insect species, the cabbage looper (*Trichoplusia ni*), southern armyworm (*Spodoptera eridania*) and black swallowtail butterfly (*Papilio polyxenes*). In general, the constitutive enzyme levels correlate well with these insects natural feeding habits and their relative susceptibility to plant pro-oxidant allelochemicals, quercetin (a flavonoid) and xanthotoxin (a photoactive furanocoumarin). An increase in SOD activity which rapidly destroys superoxide radicals, appears to be the initial response of these insects to dietary pro-oxidant exposure. The presence of high CAT activity protects these insects by destruction of H_2O_2, a cytotoxic product of SOD-catalyzed dismutation of superoxide radicals. Low GPOX activity and high GST's peroxidase activity suggests that the latter enzyme has a prominent role in scavenging deleterious LOOH's, and the GSSG generated from this reaction is reduced to GSH by GR activity. A key role for SOD was evidenced when its inhibition resulted in enhanced pro-oxidant induced toxicity.

Key words: antioxidants, black swallowtail butterfly, cabbage looper, catalase, glutathione peroxidase, glutathione reductase, glutathione-*S*-transferase, hydroxyl radical, lipid peroxidation, oxygen radicals, *Papilio polyxenes*, photosensitization, quercetin, singlet oxygen, southern armyworm, *Spodoptera eridania*, superoxide dismutase, superoxide radical, *Trichoplusia ni*, xanthotoxin.

INTRODUCTION

While the ground-state molecular oxygen ($^3O_2$) provides enormous advantages for the sustenance of aerobic life processes, it also imposes universal toxicity (Fridovich, 1983). Extensive literature has accumulated on this aspect for mammalian species, but until recently, this area was virtually unexplored for phytophagous insects. This paper summarizes the results of our recent investigations which have elucidated the mechanisms for regulating oxygen toxicity in insects.

Mechanisms of production of toxic forms of oxygen. As described previously (Ahmad et al., 1989, and refs. therein) one-electron reduction of $^3O_2$ leads to the generation of superoxide anion radical (O_2). The endogenous sources for the production of O_2 include various autoxidizable molecules, oxidoreductases and hemoproteins (Fig. 1.).

Moreover, O_2 is converted to the hydroperoxy radical (HO_2), H_2O_2 and finally to the hydroxyl radical (•OH) via the metal-catalyzed Haber-Weiss reaction. $^3O_2$ is also activated in photosensitization and other reactions to the singlet molecular oxygen ($^1O_2$).

The ubiquitous flavonoid, quercetin, and the linear furanocoumarin, xanthotoxin, are examples of many pro-oxidant plant allelochemicals which are exogenous sources of oxidative stress for phytophagous insects. Quercetin, an autoxidizable redox active molecule, generates O_2, H_2O_2 and OH radical (Hodnick et al., 1989).

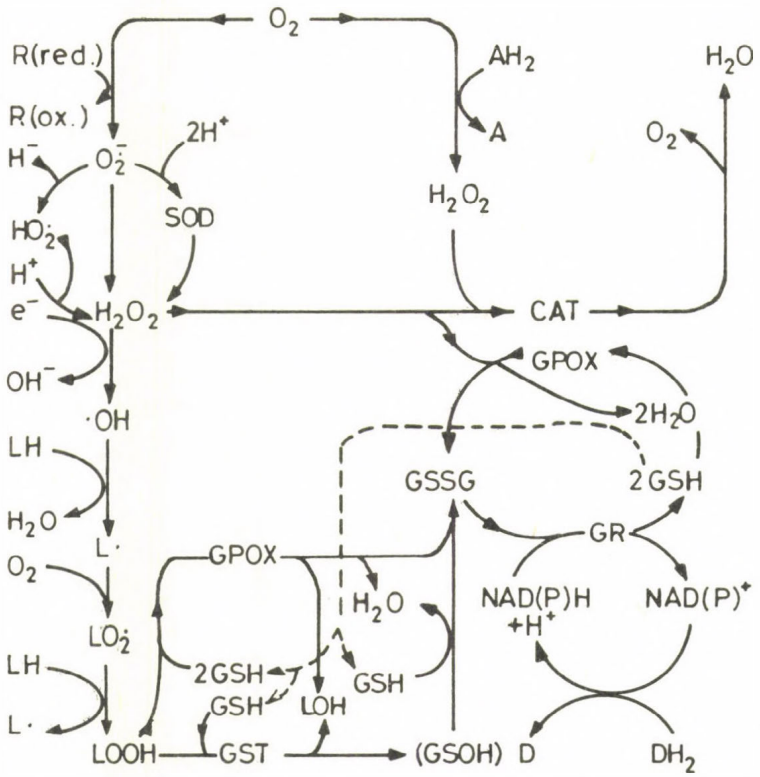

Fig. 1 General scheme for oxygen radical cascade and the roles of SOD, CAT, GPOX, GST, and GR antioxidant enzymes in regulation of oxygen toxicity in insects.
R (red.) = electron donor such as a catechol, ubisemiquinone, and metals or metalloproteins; R (ox.) = is the oxidized form of electron donors; OH = hydroxyl radical; LH = polyunsatured fatty acid, or other unsaturated organic molecules; L = lipid radical; LO_2 = lipid peroxy radical; LOOH = lipid hydroperoxide; LOH = lipid alcohol; AH_2 = A two-electron donor; A = a fully oxidized form of AH_2; GSH = reduced glutathione; GSSG = oxidized glutathione (glutathione disulfide); (GSOH) = unstable sulfenic acid of glutathione; DH_2 and D = nonspecified NAD (P) redox system.

Xanthotoxin is a photosensitizer (S) which is activated by long-UV light (320-380 mm). Its toxicity is expressed via a type I (oxygen-independent) mechanism involving deleterious interactions of the excited sensitizer (^3S) with macromolecules, and type II (oxygen-dependent) mechanism which results in the production of $^1O_2$ (Downum and Rodriguez, 1986). Moreover, O_2 is also generated by xanthotoxin, presumably, via type I mechanism analogous to photosensitive dyes (Burch and Martin, 1988) by conversion of ^3S to a free radical species which then interacts with $^3O_2$ and generates O_2, H_2O_2 and OH radical.

As depicted in Fig. 1, the OH radical can cause formation of hydroperoxides of lipids (LOOH), and peroxides of steroids and DNA. Moreover, many unsaturated organic molecules are peroxidized by the insertion of $^1O_2$.

Oxigen Toxicity. Both OH radical and $^1O_2$ are the most reactive forms of activated $^3O_2$ and react with macromolecules such as DNA, RNA and proteins and, also, are responsible for deleterious lipid peroxidation. In insects, lipid peroxidation is potentially very harmful because lipids not only are essential components of cell membranes, but also have unique physiological functions (Downer, 1986). Tissues may be directly oxidatively damaged by peroxides, or from more reactive breakdown products of peroxides, such as malonaldehyde and decomposition, by metals to regenerate the peroxidizing LO radical.

DEFENSE MECHANISMS OF INSECTS AGAINST PRO-OXIDANTS

Antioxidants. The antioxidant compounds β-carotene and α-tocopherol (vitamin E) are the only known defense mechanisms against $^1O_2$, while sulfhydryl compounds and vitamin E provide a line of defense against OH and O_2, or LO_2 radicals. In such reactions the antioxidants are destroyed, but some such as vitamin E are restored by Vitamin C. Vitamin C in turn is stabilized by urate (Hochstein et al., 1984), and urate is becoming recognized as an important antioxidant for O and peroxy radicals.

Phytophagous insect species can obtain adequate supply of these antioxidants, and they generate more copious amounts of uric acid than do the mammals, yet their precise roles have not been elucidated.

Additionally, enzymatic defenses are crucial in terminating the oxygen-radical cascade, and the removal of LOOH to terminate peroxidation chain reactions.

Pro-oxidant susceptibility. Toxicological data indicate that the pro-oxidants, quercetin and xanthotoxin, are acutely toxic to *T. ni* (Ahmad et al., 1987). *S. eridania* is more broadly polyphagous species than *T. ni*, and sublethal doses of pro-oxidants effect its relative growth rates (Pritsos et al., 1988a, 1988b). *P. polyxenes* is completely tolerant to both pro-oxidants (Pritsos et al., 1988b).

Antioxidant enzymatic defense. In general, the enzyme levels are consistent with the *P. polyxenes* high, *S. eridania*'s moderate and *T. ni*'s low tolerance to pro-oxidants. Superoxide dismutase (SOD) and catalase (CAT) levels with the ontogeny of larvae showed a marked drop as larvae advanced from mid- to late-stage (Ahmad et al., 1987; Pritsos et al., 1988a, 1988b). This pattern suggests the sequential action of

Table 1 Subcellular distribution and activities (mean units + SD) of antioxidant enzymes of phytophagous insect species. [a,b]

Antioxidant Enzyme	Insect Species	Subcellular Compartments			
		Nucleus	Mitochondria	Microsomes	Cytosol
SOD	*T. ni*	0.33±<0.01	3.05±0.11	0	0.97±0.04
	S. eridania	ND	2.13±0.13	0.78±0.02	4.26±0.11
	P. polyxenes	0.39±0.28	4.47±1.45	1.08±1.09	4.33±0.39
CAT	*T. ni*	22.9±1.21	283±41	142±31	150±18
	S. eridania	ND	125±40	119±15	163±30
	P. polyxenes	283±6	336±13	106±8	135±10
GPOX	*T. ni*	2.0±0.28	2.5±0.76	2.8±0.8	<0.2
	S. eridania	46.6±19.6	14.0±7.2	16.1±5.0	0
	P. polyxenes	ND	ND	ND	ND
GST	*T. ni*	74.4±4.3	0	34.6±2.12	13.0±0.84
	S. eridania	ND	ND	ND	ND
	P. polyxenes	308±10	63.9±18.1	46.3±6.5	10.5±1.0
GR	*T. ni*	3.68±1.21	3.68±1.00	2.46±1.08	0.70±0.21
	S. eridania	ND	5.49±0.90	3.30±0.39	0
	P. polyxenes	92±18	39.9±9.1	25.3±9.4	4.5±2.9

[a] Data are from Ahmad et al. (1988a, 1988b, 1989) and unpublished data on *P. polyxenes* (S. Ahmad, C. A. Pritsos and R. S. Pardini, 1989); ND = not determined.

[b] The procedures for assays of antioxidant enzymes were exactly as reported earlier, including the basis for expressing enzyme activities in units (Ahmad et al. 1988a, 1988b, 1989).

these enzymes for the removal of O_2 and H_2O_2. In contrast to SOD and CAT ontogeny, the glutathione reductase (GR) activity increased as larvae aged within each instar, and also from third to fifth instars.

Initially, the glutathione peroxidase (GPOX) activity detected was negligible, and nearly the same activity was found in heat-inactivated enzyme preparations. This led to the perception that this activity may be a procedural artifact and, therefore, it did not indicate the presence of this selenium-dependent enzyme. We then hypothesized that in the absence of GPOX, the insects may be relying on non-selenium-dependent glutathione-*S*-transferase's (GST's) peroxidase activity to reduce LOOH (Ahmad et al., 1988a, 1988b) but not H_2O_2. Using cumene hydroperoxide (cumOOH) as substrate, and inhibitory effects of cyanide and cysteamine, we demonstrated and distinguished GST's peroxidase activity from that of GPOX in *T. ni* larvae (Ahmad and Pardini 1988, 1989). The enzymology of GST's xenobiotic GSH-conjugative and peroxidative activities in *T. ni, S. eridania* and *P. polyxenes* has been recently characterized (L. C. Weinhold, S. Ahmad and R. S. Pardini, unpublished, 1989). The peroxidase activity of GST in midfifth-instar larvae of these insects is high; 50, 106 and 253 units, respectively, in *T. ni, S. eridania* and *P. polyxenes*.

Recently, the GPOX activity was re-investigated and confirmed. With the use of H_2O_2 as substrate, and an ironspecific chelator, desferrioxamine, the activity was found to be 2, 12.8, and 12.3 units in midfifth-instar larvae of *T. ni, S. eridania* and

P. polyxenes. These GPOX activities are considerably lower than in most mammalian tissues, i.e., in rat tissues GPOX levels range from 100-1000 units, except for muscles (20-30 units) (Ahmad et al., 1989).

The subcellular distribution of the antioxidant enzymes is summarized in Table 1. Insects' SOD activity is primarily confined to cytosol and mitochondrial matrix as in other eukaryotes. In the mammalian species, the peroxisomes are considered the normal site for CAT for the destruction of H_2O_2. A number of enzymatic processes in the peroxisomes produce H_2O_2 by a direct two-electron reduction of $^3O_2$. Therefore, the peroxisomal localization of CAT is considered to be strategic. On the other hand, the subcellular distribution of GPOX and GR in cytosol and mitochondrial matrix in mammalian species is complementary to that of CAT. GST's peroxidase activity is important in protecting endoplasmic reticulum membranes from peroxidation injury, and for repair of peroxidized DNA.

The subcellular distribution of GPOX and GR is different in insects than in mammalian species, and work on *T. ni* shows no GST's peroxidase activity in the mitochondria. The CAT activity of insects is not only very high, its subcellular distribution is also very broad (Ahmad et al., 1988a, 1988b, 1989). Because GPOX but not GST is present in *T. ni*'s mitochondria, it seems feasible that these enzymes may act in a site-specific manner to reduce H_2O_2 and LOOH's.

Generally, an increase in SOD activity appears to be a primary response of midfifth-instar larvae of our model insect species to sublethal dietary exposure to pro-oxidant compounds, quercetin and xanthotoxin. At low concentration of pro-oxidants the CAT, GPOX, GST and GR activities were not altered, but at 10-fold higher concentration their activities declined. Nonetheless, sufficient activity of all four enzymes remained for their respective catalytic activities (Pritsos et al., 1988b, S. Ahmad and R. S. Pardini, unpublished, 1989).

When the cytosolic SOD, a copper-zinc enzyme, was inhibited by diethyldithiocarbamate (DETC), quercetin toxicity dramatically increased for midfifth-instar larvae of *P. polyxenes* and *S. eridania*. DETC had no effect on quercetin toxicity in *T. ni* which has the lowest SOD activity of all three insect species (C. A. Pritsos, J. Pastore and R. S. Pardini, unpublished, 1989). Furthermore, *T. ni* has lower amounts of the cytosolic CuZn-SOD compared to higher amounts of the mitochondrial enzyme which presumably is a Mn-SOD (Ahmad et al., 1988a). These results elucidate the important role of SOD in the pro-oxidant allelochemical defense of insects.

DISCUSSION AND CONCLUSIONS

As depicted in Fig. 1 all five antioxidant enzymes known from mammalian species have been found in phytophagous insects. SOD catalyzed dismutation of O_2 radicals is 10^{10}-fold faster than the spontaneous dismutation of O or its protonated form HO_2 at the physiological pH 7.8. The rapid induction of this enzyme from O_2 generating pro-oxidants, and enhanced toxicity of pro-oxidants when the enzyme is inhibited by DETC, indicate a key role for SOD as a primary antioxidant defense

system in insects. However, the dismutase reaction catalyzed by SOD results in the production of H_2O_2, which in the presence of iron is more toxic to cells than O_2. CAT, however, reduces H_2O_2 to H_2O. These two enzymes work sequentially to efficiently reduce that portion of oxidative stress which is propagated by O_2 radicals and H_2O_2. Thus, the potential oxygen-radical cascade by these activated forms of $^3O_2$ to lipid peroxidizing LO_2 radical is, in large measure, prevented. In contrast to mammalian species, the activity of the selenium-dependent GPOX which removes H_2O_2 and LOOH is much lower in insects. High peroxidase activity of GST which reduces a wide range of peroxides might compensate for low GPOX activity. However, GST's inability to catalyze the reduction of H_2O_2 has led to another evolutionary modification. The CAT activity of insects was enhanced and its subcellular distribution was extended to cytosol and most other membrane-limited organelles. This adaptation represents an efficient protective strategy against the threat of large cellular increases of H_2O_2 under oxidative stress. The role of GR is also crucial in that it reduces GSSG which is formed during the catalytic activities of both GPOX and GST to GSH.

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REFERENCES

Ahmad, S., M. A. Beilstein and R. S. Pardini (1989): Glutathione peroxidase activity in insects: A reassessment. *Arch. Insect Biochem. Physiol.* (in press)

Ahmad, S. and R. S. Pardini (1988): Evidence for the presence of glutathione peroxidase activity toward an organic hydroperoxide in larvae of the cabbage looper moth, *Trichoplusia ni. Insect Biochem. 18,* 861-866.

Ahmad, S. and R. S. Pardini (1989): Corrigendum. *Insect Biochem. 19,* 109.

Ahmad, S., C. A. Pritsos, S. M. Bowen, K. E. Kirkland, G. J. Blomquist and R. S. Pardini (1987): Activities of enzymes that detoxify superoxide anion and related oxyradicals in *Trichoplusia ni. Arch. Insect Biochem. Physiol. 6,* 85-96.

Ahmad, S., C. A. Pritsos, S. M. Bowen, C. R. Heisler, G. J. Blomquist and R. S. Pardini (1988a): Antioxidant enzymes of larvae of the cabbage looper moth, *Trichoplusia ni:* Subcellular distribution and activities of superoxide dismutase, catalase and glutathione reductase. *Free Radical Res. Commun. 4,* 403-408.

Ahmad, S., R. S. Pardini, C. A. Pritsos, S. M. Bowen, C. R. Heisler and G. J. Blomquist (1988b): Subcellular distribution and activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in the Southern armyworm, *Spodoptera eridania. Arch. Insect Biochem. Physiol. 7,* 173-186.

Burch, P. E. and J. P. Martin Jr. (1988): Protection against dye mediated photodynamic effects is conferred by DNA repair enzymes and oxygen radical scavengers. *FASEB J.* 2:A766/2729.

Downer, R. G. H. (1986): Lipid metabolism. In: G.A. Kerkut and L.I. Gilbert (eds): *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Pergamon Press, Oxford. 77-113.

Downum, K. R. and E. Rodriguez (1986): Toxicological action and ecological importance of plant photosensitizers. *J. Chem. Ecol. 12,* 823-834.

Fridovich, I. (1983): Superoxide radical: an endogenous toxicant. *Ann. Rev. Pharmacol. 23,* 239-257.

Hochstein, P., L. Hatch and A. Boveris (1984): Uric acid: Functions and determinations. *Meth. Enzymol. 106,* 162-166.

Hodnick, W. F., B. Kalyanaraman, C. A. Pritsos and R. S. Pardini (1989): The production of hydroxyl and semiquinone free radicals during the autoxidation of redox active flavonoids. In: M. G. Simic, K. A. Taylor, J. F. Ward and C. von Sonntag (eds): *Oxygen Radicals in Biology and Medicine*. Plenum Press, New York, 149-152.

Pritsos, C. A., S. M. Ahmad, S. M. Bowen, G. J. Blomquist and R. S. Pardini (1988a): Antioxidant enzyme activities in the southern armyworm, *Spodoptera eridania. Comp. Biochem. Physiol. 90C,* 423-427.

Pritsos, C. A., S. Ahmad, S. M. Bowen, A. J. Elliott, G. J. Blomquist and R. S. Pardini (1988b): Antioxidant enzymes of the black swallowtail butterfly, *Papilio polyxenes,* and their response to the prooxidant allelochemical, quercetin. *Arch. Insect Biochem. Physiol. 8,* 101-112.

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PERCEPTION OF SURFACE CHEMICALS BY FEEDING AND OVIPOSITING INSECTS

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ABSTRACT

The methods are reviewed which were used to extract the plant surface yielding compounds influencing the behavior of herbivore insects. The classical solvents employed so far were methylene and chloroform in which undamaged leaves were dipped. In the case of cabbage leaves the authors obtained little or no stimulatory activity using these procedures. New methods based on hot water or a sequence of dippings chloroform and methanol were developed producing extracts stimulating oviposition in the cabbage root fly, *Delia radicum*, and other insects.

Behavioral patterns involved in the exploration of the leaf surface and its boundary layer are reviewed. These include olfaction prior to landing, palpation, tapping with the proboscis and antenna, drumming with the prothorax tarsi, running on the leaf, and probably finally also ovipositor contact.

In the perception of the leaf surface chemicals both contact and olfactory chemoreceptors are involved. In the carrot fly, *Psila rosae*, the receptors on the antennae were shown to be selectively sensitive to stimulatory compounds isolated from the carrot leaf surface. In the black swallowtail contact chemoreceptor sensilla on the proximal surface of the prothorax tarsi receptor cells have been found to be sensitive to host and non-host surface leaf extracts and to a stimulating compound isolated from carrot leaves, luteolin 7-*O*-(6"-*O*-malonyl)-β-D-glucopyranoside.

Key words: leaf surface chemicals, leaf surface wax, extraction, boundary layer, luteolin 7-*O*-(6"-*O*-malonyl)-β-D-glucopyranoside, falcarindiol, exploration behavior, feeding, oviposition, olfaction, contact chemoreception, antenna, tarsus, *Delia radicum, Papilio polyxenes, Psila rosae*, Cruciferae, Umbelliferae, *Brassica oleracea, Daucus carota.*

INTRODUCTION

The fact that the plant surface is an important "interface" between phytophagous insects and the host plant is gaining more and more attention in the research of plant insect relationships. Recently this subject has been reviewed by Chapman and Bernays (1989) and Woodhead and Chapman (1986) for feeding insects and by Städler (1986) regarding ovipositing females. We will therefore concentrate on the newer publications and unpublished data from our laboratory. Special issues will be the methodology of studying plant chemicals occurring on the leaf surface, behavioral patterns which seem to be related to the perception of the leaf surface and review of our knowledge about the chemoreceptors involved.

METHODS OF EXTRACTION AND IDENTIFIED COMPOUNDS FROM PLANT SURFACE

Review: Juniper and Jeffree (1983) described the classical methods of extracting plant compounds from the leaf surface without damaging the leaf interior. Since the waxy surface of many plants contains a mixture of polar and non-polar constituents, solvents must be chosen which dissolve both. The authors therefore recommended short immersions of 10 to 30 seconds in chloroform ($CHCl_3$). Following this idea, Städler and Buser (1984) using two immersions of 30 s in methylene chloride (CH_2Cl_2), were able to extract oviposition stimulants from the carrot leaf surface. CH_2Cl_2, which has a lower boiling point than $CHCl_3$, was used to prevent loss of volatile plant compounds during concentration of the large volumes of solvents necessary for dipping.

Woodhead et al. (1982) extracted feeding deterrents for the locust from the epicuticular wax of *Sorghum bicolor*. They obtained the active extracts by immersion of intact seedlings in $CHCl_3$ for short periods and were able to identify p-hydroxybenzaldehyde as a major deterrent important for seedling resistance in acridids. Al Rouz and Thibout (1988) extracted feeding stimulants for the larvae of the leek moth *Acrolepiopsis assectella* washing *Allium* leaves with CH_2Cl_2 or MeOH. Jackson et al. (1984) successfully isolated and identified oviposition stimulants from tobacco leaves using CH_2Cl_2. Nottingham et al. (1989a) also used CH_2Cl_2 (10 s immersion of leaves of the sweet potato leaf surface, *Ipomoea batatas*, to extract attractive compounds for the sweet potato weevil *Cylas formicarius elegantulus*. Sesquiterpenes were found to be the active components. Further, Nottingham et al. (1989b) and Wilson et al. (1988) extracted the root periderm of the sweet potato, *Ipomoea batatas*, using CH_2Cl_2 in an ultrasonic bath for 9 min. These authors tentatively identified triterpeneol acetate as an oviposition stimulant for the sweet potato weevil. Harris et al. (1990) showed that the acceptance of the oviposition site by the hessian fly, *Mayetiola destructor*, is mediated by $CHCl_3$ foliar extracts of wheat leaves, the host plant, acting together with color and tactile stimuli. Juvik et al. (1988) compared different solvents for the extraction of the surface of wild tomato leaves and found that hexane extracts were the most stimulatory. Using this solvent, these investigators (Coates et al., 1988; Juvik et al., 1988) tentatively identified sesquiterpenes to be the oviposition stimulants for *Heliothis zea*.

Very polar stimulating compounds for females of *Ostrinia nubilalis* have been extracted from various leaf surfaces by Derridj et al. (1987, 1990) using (cold) distilled water. Correlations between the extract content and the oviposition preference were found, indicating that fructose was probably one of the major stimulating compounds. Also, relatively polar compounds have been extracted from the leaves of apple trees by Richmond and Martin (1959) using ethyl ether dips of 10 s duration. One of the isolated substances was phloridzin, a dihydrochalcone glycoside, which is known to influence the behavior of different insects (references in Städler, 1986).

Compounds emanating from the undamaged leaf surface have been analysed by extracting the headspace air of plants. In this manner, Finch (1978) identified iso-

thiocyanates in the headspace of cabbage plants. These compounds have been found to be attractive and synergistic to non volatiles in many insects attacking crucifers. It seems evident that these and other plant volatiles will be saturating the boundary layer on the leaf surface (Juniper and Southwood, 1986). Such odorants may also have an effect on some distance. This was shown by Tingle et al. (1989), who found that mated female moths of *Heliothis subflexa* were flying towards fresh whole-leaf wash extracts with MeOH (30 s) of the host plant, groundcherry (*Physalis*). The uninjured leaf surface and its volatiles are certainly of primary importance but compounds from plant tissue damaged by insects can not be dismissed. Hausmann and Miller (1989) presented such evidence showing the attractive properties of onions attacked by maggots of the onion fly for the female of the same species. This preference is adaptive because larval survival was higher on damaged than undamaged bulbs.

A specific source for allelochemicals influencing the behavior of insects on the leaf surface are the trichomes. Headspace compounds originating from tobacco leaf trichomes have been isolated and identified by Andersen et al. (1988). Probably the surface extracting methods using dipping will also yield substances from glandular trichomes. Special extracting procedures for trichome exudates have been described by different authors. Juvik et al. (1988) rubbed cotton over the tomato leaf surface and extracted it with hexane. The isolated sesquiterpenes stimulated the oviposition of *Heliothis zea*. Lin et al. (1987) used a glass probe to rupture the glandular trichomes and transferred the content to the solvent isooctane. The identified odd-chained ketones have been found to be repellent or toxic for different insects (references in Dimock and Tingey, 1988). Another interesting technique was used by Avé et al. (1987) who collected exudates by breaking the glandular trichome lobes of *Solanum berthaultii* and *S. tuberosum* with a capillary filled with carbon disulfide. The authors identified different volatile substances including sesquiterpenes which are repellent to aphids alighting on the leaf surface.

Leaf surface extracts of Brassica oleracea. Following the discovery that the cabbage root fly, *Delia radicum*, has contact chemoreceptors on its tarsi sensitive to specific host plant compounds (Städler, 1978), Städler and Freuler (1978, unpubl.) tried to use $CHCl_3$ and MeOH to extract the leaf surface of cabbage. Neither extract contained any compounds stimulating oviposition. It was concluded that this extraction procedure was not suitable and that the assumed stimulants were either very volatile, in an unsoluble form or very unstable. Städler, Renwick and Schöni (1985, unpubl.) attempted again to extract activity from cabbage leaves using solvents with different polarities. The oviposition experiments using *Pieris rapae* in the USA and *Delia radicum* in this laboratory revealed that hot water surface extracts were active for both species (Renwick and Städler, 1985, unpubl.), whereas all tested cold solvent extracts were not.

These preliminary results led us to investigate different extraction procedures for the cabbage leaf surface. The aim was to extract the most stimulatory activity with

the least damage to the leaf. As the results of three oviposition experiments with *Delia radicum* show (Fig. 1), we confirmed that $CHCl_3$ surface extracts applied on plastic surrogate leaves (Schöni et al., 1987; Städler and Schöni, 1989) stimulated poorly even at 20 g leaf equivalents (gle). In contrast, the hot water surface extract both at 20 and 2 gle proved to be strongly stimulatory.

Given the possible detrimental effects of boiling water, we investigated other possibilities which resulted in the following method: Leaves were extracted by dipping for 5 s each in 1) $CHCl_3$ [2.0 l/kg leaf], 2) MeOH [2.5 l/kg leaf] and 3) MeOH [2.5 l/kg leaf]. Between dippings the leaves were in the air for 10 s. Special care was taken to prevent damage to the leaves during dipping. The solvents were concentrated under vacuum, centrifuged (5000 rpm, 20 min) and filtered through a glass filter paper to remove insoluble particles and evaporated to a small volume. A preliminary comparison of the MeOH extract and a hot H_20 extract showed that both were active [Fig. 2(1)]. We compared the resulting $CHCl_3$ extract with the combined MeOH extracts [Fig. 2(2)] at a concentration of 3 gle applied on surrogate leaves made of waxed paper (Roessingh and Städler, 1990). As can be seen from the egg counts, the MeOH fraction was active, and again much more stimulatory than the $CHCl_3$ extract.

To compare the activity of the MeOH surface extract with whole leaf extracts (Schöni et al., 1987), we produced a total leaf extract and a surface extract from 200 g of matched leaves from the center of cauliflower plants *(Brassica oleracea* L. convar. *botrytis* CC Cross). For the total leaf extract we employed the procedure developed by Nielsen (1988, unpubl. results): 200 g of cauliflower leaves were frozen in liquid nitrogen, powdered, and lyophilized. The leaf powder was poured into 1 l boiling

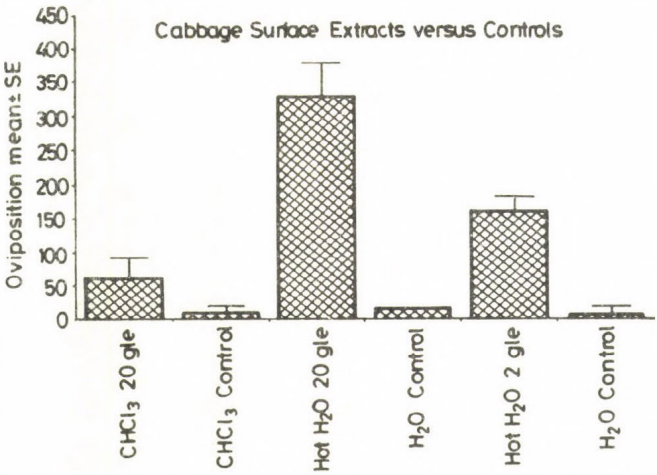

Fig. 1 Oviposition of the cabbage root fly: Comparison of 3 different surface extracts with controls.

Table 1 Comparison of the stimulatory activity of total cabbage leaf extract with surface extract.

Type of extraction	Dry matter of 200 g leaves (gle)	Applied per surrogate leaf		N	Mean	SE
		gle	dry matter			
Total MeOH extract	4.08 g	0.5	10.2 mg	8	94.38	20.02
Surface MeOH extract	0.56 g	3.65	10.2 mg	8	107.75	17.36

Applied at same amount of dry matter per surrogate leaf.
Difference between the means is not significant

70% MeOH standing on a hot plate and homogenized for 5 min. The resulting slurry was filtered through a glass filter and the residue was reextracted 2 times. The combined extract in 3 l of MeOH was concentrated under vacuum, centrifuged and filtered. Aliquots of both the surface extracts and the total leaf extract were dried and weighed. For the comparison the two extracts were applied on surrogate leaves at equal dry matter content, at a concentration of 0.5 gle for the total leaf extract and of 3.65 gle for the surface extract. The two extracts were found to stimulate oviposition as expected (Table 1). These data show that we found no significant difference at equal concentrations of dry matter, although the surface extract required about 7.3 times as much starting material to obtain the same amount of dry matter. Since the surface extract is probably less complex, we plan in the future to analyse the methanolic surface extracts.

Alborn et al. (1985) studied cabbage leaf surface compounds stimulating the turnip root fly, *Delia floralis*. Independent of our study, the authors found that condensed surface washings obtained by blowing steam over the leaves were stimulatory for this related fly. Obviously the steam (hot water) did melt and wash off stimulating compounds from the leaf surface as in our experiment. The question arises why extracting solvents in general and cold water in particular contain little or no oviposition stimulants, whereas hot water extracts and methanol extracts (after removal of the

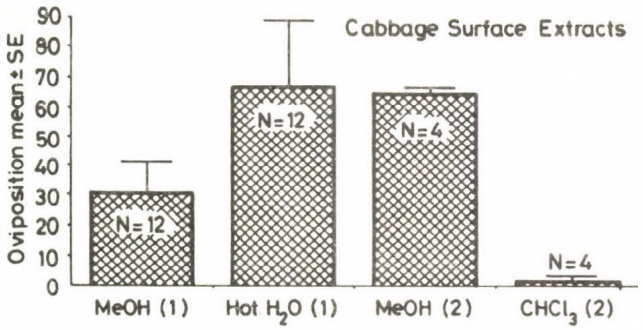

Fig. 2 Oviposition of the cabbage root fly: Comparison between surface extracts. (1) Experiment 1: MeOH versus hot H_2O extract. (2) Experiment 2: MeOH versus $CHCl_3$.

mostly non active wax layer with $CHCl_3$) do contain stimulants. Both $CHCl_3$ and hot water dissolve or melt the surface wax layer and seem to release stimulating compounds. Because the cabbage root flies and the turnip root flies can perceive them, they must somehow be exposed on the surface or be present in the boundary layer as volatiles. Since the stimulatory compounds in the surface extract are not very volatile (Roessingh et al., unpubl.) the last hypothesis is unlikely. Therefore, it seems that the stimulants are incorporated into the wax matrix of the surface, which makes them hard to dissolve, but still accessible for the chemoreceptors of the insects. To our present knowledge (Schöni et al., 1987; Roessingh et al., unpubl.), the oviposition stimulants for the cabbage root fly must be very polar and the waxes are known to be completely nonpolar. It can be speculated therefore that some type of interphase between the waxes and the stimulating compounds must exist.

Hot water extracts of other leaf surfaces. Zobel and Brown (1988) also successfully used hot H_2O to extract furanocoumarins from the leaf surface of *Ruta graveolens* and showed using this method that the yield was one to three orders of magnitude better than the cold organic solvent extracts. The tests of the authors failed to reveal any significant leakage from the interior of the leaf due to cell disruption. But the enhanced recoveries were due to damage to a deeper layer of the cuticle which apparently released the coumarins. Since insects do not damage the leaf prior to feeding, this hot H_2O extract probably contains more compounds than can be perceived. An obvious disadvantage of any heat treatment is possible breakdown of active labile compounds. In addition, since large volumes of water necessary for the dipping cannot easily be concentrated by evaporation, we decided to continue our investigation of the cabbage leaf surface using the MeOH extraction following an initial $CHCl_3$ extraction.

Conclusion. Our experience and the reports from the available literature show that different extraction techniques may yield active compounds from the leaf surface. It seems that no generalizations can be made *a priori* as to which of the techniques may be most successful for a particular plant and a phytophagous insect. Further, this holds true for attractants, stimulants, repellents or deterrents. The many publications in recent years show that active compounds are indeed present on the leaf surface and can be extracted, isolated and identified. Single active compounds can not be expected in any chemical plant-insect relationship. Synergistic effects between stimulants or deterrents and inhibitory effects between stimulants and deterrents can be expected. Leaf surface compounds, as has been shown for the carrot rust fly (Städler and Buser, 1984; Städler, 1986), are no exception. With regard to the chemical characteristics of the compounds influencing insect behavior it seems clear that compounds of different plant metabolic origin (primary and secondary) are involved. Despite the primarily non-polar epicuticular wax, very polar compounds, like sugars and amino acids, are present on the leaf (Juniper and Jeffree, 1983; Juniper and Southwood, 1986). As the experiments of Derridj et al. (1987, 1990), with the oviposition preference of *Ostrinia nubilalis* suggest, these primary plant compounds play a role in insect host plant selection. In parallel with this, Chapman and Bernays (1989) believe that "there is good evidence that host-plant recognition by oligopha-

gous insects does not depend on the presence of secondary chemical (note that only phagostimulants are meant) characteristics of the host taxa, and in these cases chemicals on the leaf surface are important phagostimulants". This statement is based on behavior associated with feeding and excludes olfaction or compounds produced by glandular trichomes. Indeed with the exception of *Pieris* cited by the authors there seems to be no direct evidence which would be in contradiction. Secondary compounds on the leaf all seem to be involved in deterring or repelling feeding insects which seems to support the hypothesis (Jermy and Szentesi, 1978; Jermy, 1983) that these compounds play a major role in host plant selection. Since these conclusions definitely do not apply to oviposition behavior, we are inclined to believe that conclusions about phagostimulants also must still be regarded as tentative because our knowledge about the relevant chemistry of the leaf surface is still fragmentary. However, because in most insects the ovipositing females select the host plant and the larvae have only a limited capacity to move and to locate host plants, it seems likely that some marked differences between the phagostimulants for adults and larvae could exist.

BEHAVIORAL PATTERNS INVOLVED IN THE EXPLORATION OF THE UNDAMAGED PLANT SURFACE AND RECEPTORS INVOLVED

Chapman and Bernays (1989) stated "critical observation of a number of different insect species show that before feeding on a plant they explore its surface". Based on our experience we fully agree with this statement and we try to add further examples to the studies cited by these authors. The exploratory behavior is obviously involved in the discrimination between host and non-host plants, but probably equally important is the location of the most suitable host plant or the best host plant tissue. In fact it is known (Juniper and Jeffree, 1983; Juniper and Southwood, 1986) that the epicuticular wax composition can vary with the orientation of the leaf and its physiological condition (age, illumination, nutrition). Maloney et al. (1988) have shown that such "plant surface information" varied between host tree foliage and influenced the stimulation of feeding.

Prior to contact. Probably most phytophagous insects are able to make some discrimination between host and non host plants already before landing. On the antennae of many insects, specific receptors have been located and identified (Visser, 1986, 1988) which are sensitive to compounds of the leaf surface (Frey et al., 1990). Special exploratory behavior in the vicinity of the host plant has been described for butterflies (for review see Feeny et al., 1983, 1989). Female *Papilio polyxenes* flutter or fan with their wings before and after landing on the host plant (Umbelliferae). Since these wing movements at zero ground speed produce a strong air current to the insect and since the antennae are bent forward with most of the olfactory sensilla exposed, this behavior could be related to perception of the plant odor ("sniffing"). Evidence for such a function is the fact that the antennae of this butterfly do indeed perceive host plant volatiles from the leaf surface. Further it has been shown that

these volatile compounds synergize the action of non-volatiles perceived upon contact with the leaf surface (Feeny et al., 1989).

Palpation. Chapman and Bernays (1989) and Städler (1986) cited many detailed observations showing that prior to feeding the plant surface is examined by many if not all insects with the (maxillary) palpi and in some cases also with the antennae. Chapman and Bernays (1989) point to the important fact that palpation is often overlooked because the insects have been deprived of food too long or because no short-term behavioral observations are done.

Investigations of the receptors involved in palpation are still very rare. The first evidence that compounds from the leaf surface are perceived by the receptors on the tip of the palps of *Locusta migratoria* has been presented by Bernays et al. (1975, 1976) and Blaney and Duckett (1975).

Tapping with proboscis. In Homoptera, which lack palps, receptors on the proboscis seem to be involved in the exploratory behavior. Ejection of saliva on the leaf surface and later reimbibition may also be involved in the perception of compounds on the leaf surface prior to insertion of the stylets (Chapman and Bernays, 1989). For other insects little evidence has been presented that proboscis receptors are involved in the exploration of the leaf surface. It is known of course that many adult insects extend their proboscis regularly while walking over any surfaces. Therefore Städler and Seabrook (1974) tried to record from the proboscis receptors of the moth *Choristoneura fumiferana* and failed to find a specific reaction of the receptor cells to the contact with host needles. More recently, Rivet and Albert (1989) using amputations came to the conclusion that the receptors on the proboscis are indeed involved in host plant selection prior to oviposition. In the cabbage root fly, Städler (1978) failed to record specific responses from the proboscis sensilla to a glucosinolate. Similar attempts in the carrot fly using identified compounds (Städler and Buser, 1984; Städler 1986) also gave no indication for specific receptors (Städler, unpubl.). When more active compounds are isolated and identified, further investigations of proboscis receptors will be possible and will give a more definitive view of the role of these sensilla in leaf surface exploration.

Drumming. It is well known that butterflies drum the leaf surface prior to oviposition (references in Städler, 1986). Several investigators speculated that perhaps the leaf surface wax is damaged during this behavior, allowing the tarsal sensilla to contact compounds from deeper in the epicuticule. However, Traynier and Hines (1987) failed to find any damage to cabbage leaves drummed by *Pieris rapae*. Similarly Calvert and Hanson (unpubl.) inspected leaf surfaces that had been drummed by *Chlosyne* females and could not find any signs in SEM pictures. However Boppré (1983) found that Danaidae butterflies are able to scratch the leaves with their foretarsi to get access to the pyrrolizidine alkaloids of *Heliotropium pectinatum.*

We have started an investigation of the tarsal sensilla of *Papilio polyxenes* using extracts and pure compounds which have been isolated from one of its host plants by Feeny and collaborators (Feeny et al., 1983, 1988). The sensilla of the prothoracic tarsi of this butterfly contain receptors for host and non host-plant compounds. Fig. 3 shows representative recordings from a sensillum stimulated with methanolic sur-

face extracts, the host plant (carrot) and a non-host (cabbage). The control (KCl 10 mM) did not show any activity. Carrot and cabbage extracts produced reproducible and concentration-dependent spike patterns. The carrot extract always elicited two or three cells, as in the example shown in Fig. 3. In contrast, cabbage extracts elicited the firing of one receptor cell with large size spikes, with only a few of one or two types of smaller spikes. Recently Feeny et al. (1988) isolated and identified the first

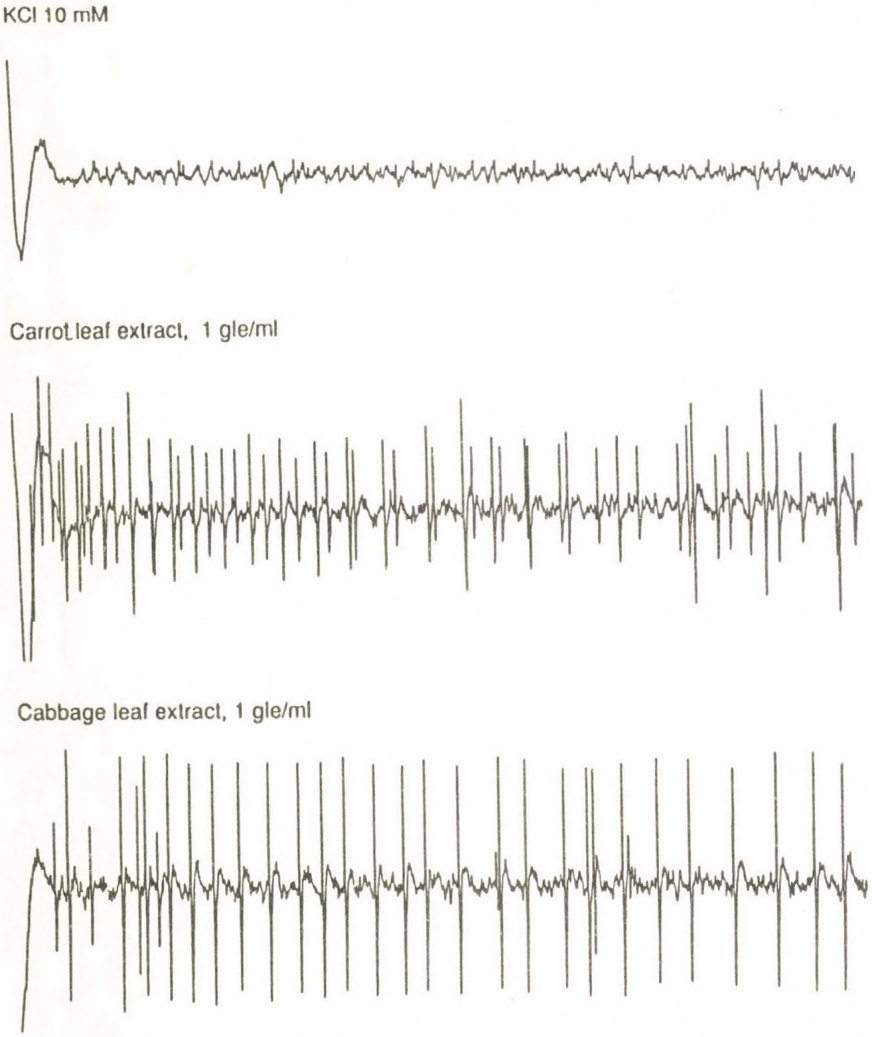

Fig. 3 Recordings of stimulations with leaf extracts from one tarsal sensillum on the prothorax tarsus of the black swallowtail: Duration 1 s starting with contact artefact. Concentration in gle (gram leaf equivalent).

oviposition stimulants from raw extracts of carrot leaves. We used this glycoside, luteolin 7-*O*-(6"-*O*-malonyl)-β-D-glucopyranoside, to stimulate the same sensilla that are sensitive to the carrot extract. As can be seen from the example of Fig. 4 we found sensilla containing a receptor cell sensitive to this compound. Typically all receptor cells that we identified had a pronounced phasic response to the stimulation. We concluded that the chemoreceptors in the tarsal sensilla can perceive plant

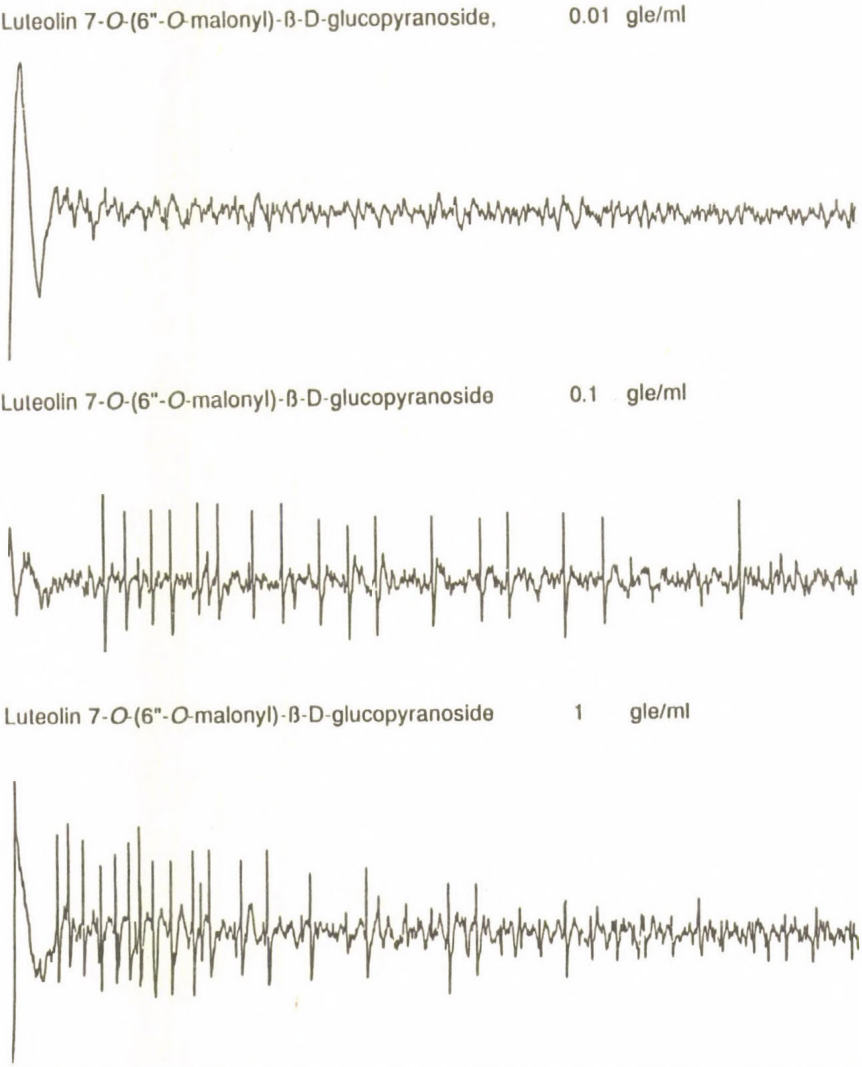

Fig. 4 Recordings of stimulations with pure Luteolin 7-*O*-(6"-*O*-malonyl)-β-D-glucopyranoside from one tarsal sensillum on the prothorax tarsus of the black swallowtail: Duration 1 s starting with contact artefact. Concentration in gle carrot leaf extract.

compounds and could allow the butterfly to discriminate between carrot and cabbage. Using this glycoside and other compounds which will be identified in the future we hope to analyse the complex recordings obtained from the total carrot extract. Thus we hope to analyse the complex code of the different receptor cells with the help of the individual components producing it.

Exploratory running. Städler (1986) already compared palpation and drumming with the running of different female insects on their host plants. Städler (1978) found that the cabbage root fly *Delia radicum* has some sensilla on its tarsi responding to glucosinolates. Recent recordings (Städler et al., unpubl) revealed that these sensilla have also receptors for plant extracts. Since the continual contact with the extract-treated leaf model is important for normal oviposition behavior (Städler and Schöni, 1989) and since active main compounds of the extracts are not volatile (Roessingh et al., unpubl.) it seems that the tarsal receptors must be involved in the perception of the leaf surface.

In the carrot rust fly, *Psila rosae*, which shows a preoviposition behavior similar to that of the cabbage root fly (Städler, 1986), we have not been able to find a receptor cell in the tarsal sensilla sensitive to the identified oviposition stimulants of the leaf surface. This prompted us to investigate the sensory response of the antennae to these compounds. As can be seen from the EAG data in Fig. 5, we were able to record evoked potentials from all the important compounds of the carrot leaf surface: phenyl propanoids (trans asarone), furanocoumarins (bergapten, osthol) and polyacetylenes (falcarindiol, falcarinol, and falcarinon). The assumed biosynthetic precursors of the active polyacetylenes (elaidinic acid, oleic acid) were far less active,

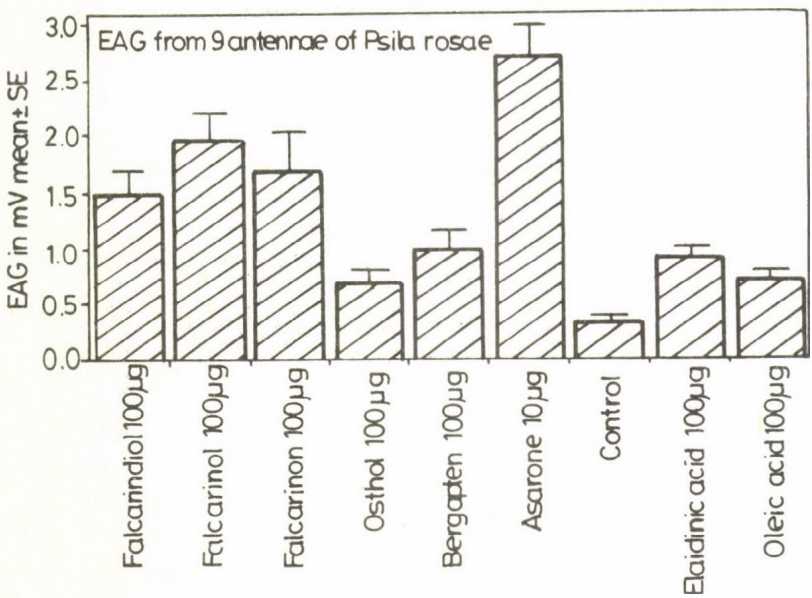

Fig. 5 Electroantennograms (EAG) from the carrot fly: Volatiles were applied in an airstream with a pulse from a pasteur pipette containing the compound.

showing that the olfactory receptors in the antennal sensilla must have some specificity. Trans asarone and the related methylisoeugenol have been shown by Guerin et al. (1983) to be attractive in the field. In contrast, we have not been able to show the same for the other compounds of the CH_2Cl_2 surface extract from carrot leaves. Since furanocoumarins and polyacetylenes have a relatively high vapor pressure this is not too surprising. We believe, therefore, that these compounds are only perceived very close to the host plant. The boundary layer over the leaf which is about 1 mm thick will be saturated with these compounds (Juniper and Southwood, 1986), and should be an ideal place for the carrot fly to detect with its antennae these host plant compounds. We propose therefore that the "oviposition runs" of the different flies may allow the perception of non volatile compounds on the surface as well as volatiles in the saturated boundary layer.

Ovipositor contact. Waladde et al. (1985) identified receptors on the ovipositor of the *Sorghum* stem borer that respond to exudates of maize cultivars and to sucrose. Waladde and Ochieng (1990) report in addition that contact chemoreceptors on the ovipositor of the legume podborer respond to leaf washes. Thus, both insects seem to have the ability to discriminate leaf surfaces still in the final stage of host selection, that is during oviposition on the leaf surface. Klijnstra and Roessingh (1986) also identified contact chemoreceptor sensilla on the ovipositor of *Pieris brassicae*. However these authors found no indications that the chemoreceptor cells reacted to glucosinolates of the host plant. More behavioral data and electrophysiological experiments are clearly needed to understand the possible role of chemoreceptors on the ovipositor.

FUTURE INVESTIGATIONS

We would like to stress that for the understanding of the role of the leaf surface many more isolations and chemical identification have to be performed. This is not an easy or a fast process, but is essential for understanding the first step in the host selection. The identification of these compounds will also be a key to understanding the chemoreceptors involved in host selection and the sensory coding of the complex host plant surface signature.

We would also like to draw attention to the many microorganisms which exist on the epicuticule (phyllosphere, phylloplane flora, Juniper and Jeffree, 1983). These organisms can be very specific for a plant species and may have a role in the insect plant interaction. This should be considered when surfaces are extracted and changes of compounds leaking out on the surface are investigated.

Problems remaining for future research are the identification of epicuticular plant compounds and their pattern of distribution, as well as a better knowledge of the appropriate chemoreceptors, including how these molecules enter the sensillar pores during the brief contact with the leaf. Specific staining procedures for the active compounds may help to study their distribution and interaction with the organisms on the surface.

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REFERENCES

Alborn, H., H. Karlsson, L. Lundgren, P. Ruuth and G. Stenhagen (1985): Resistance in crop species of the genus *Brassica* to oviposition by the turnip root fly, *Hylemya floralis*. *Oikos 44*, 61-69.

Al Rouz, H. and E. Thibout (1988): Premières observations sur le comportement alimentaire des larves de la teigne du poireau, *Acrolepiopsis assectella*, rôles des Allium. *Oecol. Appl. 9*, 261-273.

Andersen, R. A., T. R. Hamilton-Kemp, J. H. Loughrin, C. G. Hughes, D. F. Hildebrand and T. G. Sutton (1988): Green leaf headspace volatiles from *Nicotiana tabacum* lines of different trichome morphology. *J. Agric. Food Chem. 36*, 295-299.

Avé, D. A., P. Gregory and W. M. Tingey (1987): Aphid repellent sesquiterpenes in glandular trichomes of *Solanum berthaultii* and *S. tuberosum*. *Entomol. exp. appl. 44*, 131-138.

Bernays, E. A., W. M. Blaney, R. F. Chapman and A. G. Cook (1975): The problems of perception of leaf-surface chemicals by locust contact chemoreceptors. In: D. A. Denton and J. P. Coghlan (eds), *Olfaction and Taste V., Academic Press, New York. 227-229.*

Bernays, E. A., W. M. Blaney, R. F. Chapman and A. G. Cook (1976): The ability of *Locusta migratoria* L. to perceive plant surface waxes. *Symp. Biol. Hung. 16*, 35-40.

Blaney, W. M. and A. M. Duckett (1975): The significance of palpation by the maxillary palps of *Locusta migratoria* (L.): An electrophysiological and behavioural study. *J. Exp. Biol. 63*, 701-712.

Boppré, M. (1983): Leaf-scratching – a specialized behaviour of Danaine butterflies (Lepidoptera) for gathering secondary plant substances. *Oecologia 59*, 414-416.

Chapman, R. F. and E. A. Bernays (1989): Insect behavior at the leaf surface and learning as aspects of host plant selection. *Experientia 45*, 215-222.

Coates, R. M., J. F. Denissen, J. A. Juvik and B. A. Babka (1988): Identification of α-santalenoic and endo-β-bergamotenic acids as moth oviposition stimulants from wild tomato leaves. *J. Org. Chem. 53*, 2186-2192.

Derridj, S., V. Fiala and E. Jolivet (1987): Low molecular carbohydrates of *Zea mays* L. leaves and the egg-laying of *Ostrinia nubilalis* Hbn. Lepid. Pyralidae. In: V. Labeyrie, G. Fabres and D. Lachaise (eds), *Proc. 6th Int. Symp. Insect-Plant Relationships, Pau 1986.* W. Junk, Dordrecht. 295-299

Derridj, S., V. Fiala and J. P. Boutin (1990): Host plant oviposition preference of the european corn borer (*Ostrinia nubilalis* Hbn.) and a biochemical explanation. (This volume)

Dimock, M. B. and W. M. Tingey (1988): Host acceptance behaviour of Colorado potato beetle larvae influenced by potato glandular trichomes. *Physiol. Entomol. 13*, 399-406.

Feeny, P., L. Rosenberry and M. Carter (1983): Chemical aspects of oviposition behavior in butterflies. In: S. Ahmad (ed), *Herbivorous Insects*. Academic Press, New York. 27-76.

Feeny, P., K. Sachdev, L. Rosenberry and M. Carter (1988): Luteolin 7-*O*-(6"-*O*-malonyl)-β-D-glucoside and trans-chlorogenic acid: oviposition stimulants for the black swallowtail butterfly. *Phytochem. 27*, 3439-3448.

Feeny, P., E. Städler, I. Åhman and M. Carter (1989): Effects of plant odor on oviposition by the black swallowtail butterfly, *Papilio polyxenes* (Lepidoptera: Papilionidae). *J. Insect Behav.* (in press)

Finch, S. (1978): Volatile plant chemicals and their effect on host plant finding by the cabbage root fly (*Delia brassicae*). *Entomol. exp. appl. 24*, 350-359.

Frey, J. E., T. J. Bierbaum and G. L. Bush (1990): Differential antennal sensitivity to host odor compounds in *Rhagoletis* sibling species (Dipt.: Tephritidae). (This volume)

Guerin, P. M., E. Städler and H. R. Buser (1983): Identification of host plant attractants for the carrot fly, *Psila rosae*. *J. Chem. Ecol. 9*, 843-861.

Harris, M. O., S. Rose and P. Malsch (1990): Egglaying behavior in the hessian fly, an ephemeral insect herbivore. (This volume)

Hausmann, S. M. and J. R. Miller (1989): Ovipositional preference and larval survival of the onion maggot (Diptera: Anthomyiidae) as influenced by previous maggot feeding. *J. Econ. Entomol. 82*, 426-429.

Jackson, D. M., R. F. Severson, A. W. Johnson, J. F. Chaplin and M. G. Stephenson (1984): Ovipositional response of tobacco budworm moths (Lepidoptera: Noctuidae) to cuticular chemical isolates from green tobacco leaves. *Environ. Entomol. 13*, 1023-1030.

Jermy, T. (1983): Multiplicity of insect antifeedant in plants. In: D. L. Whitehead and Bowers, W. S. (eds), *Natural Products for Innovative Pest Control.* Pergamon Press, Oxford. 223-236.

Jermy, T. and Á. Szentesi (1978): The role of inhibitory stimuli in the choice of oviposition site by phytophagous insects. *Entomol. exp. appl. 24*, 458-471.

Juniper, B. E. and C. E. Jeffree (1983): *Plant Surfaces*. Edward Arnold, London.

Juniper, B. E. and R. Southwood (1986): *Insects and the Plant Surface*.Edward Arnold, London.

Juvik, J. A., B. A. Babka and E. A. Timmermann (1988): Influence of trichome exudates from species of *Lycopersicon* on oviposition behavior of *Heliothis zea* (Boddie). *J. Chem. Ecol. 14*, 1261-1278.

Klijnstra, J. W. and P. Roessingh (1986): Perception of the oviposition deterring pheromone by tarsal and abdominal contact chemoreceptors in *Pieris brassicae*. *Entomol. exp. appl. 40*, 71-79.

Lin, S. Y. H., J. T. Trumble and J. Kumamoto (1987): Activity of volatile compounds in glandular trichomes of *Lycopersicon* species against two insect herbivores. *J. Chem. Ecol. 13*, 837-850.

Maloney, P. J., P. J. Albert and A. P. Tulloch (1988): Influence of epicuticular waxes from white spruce and balsam fir on feeding behavior of the eastern spruce budworm. *J. Insect Behav. 1*, 197-208.

Nottingham, S. F., K.-C. Son, D. D. Wilson, R. F. Severson and S. J. Kays (1989a): Feeding and oviposition preferences of sweet potato weevils, *Cylas formicarius elegantulus* (Summers), on storage roots of sweet potato cultivars with differing surface chemistries. *J. Chem. Ecol. 15*, 895-903.

Nottingham, S. F., K.-C. Son, R. F. Severson, R. F. Arrendale and S. J. Kays (1989b): Attraction of sweet potato weevils, *Cylas formicarius elegantulus* (Summers), (Coleoptera: Curculionidae), to sweet potato leaf and root volatiles. *J. Chem. Ecol. 15*, 1095-1106.

Richmond, D. V. and J. T. Martin (1959): Studies on plant cuticle. III. The composition of the cuticle of apple leaves and fruits. *Ann. appl. Biol. 47*, 583-592.

Rivet, M.-P. and P. J. Albert (1989): Oviposition behavior in spruce budworm *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). *J. Insect Behav.* (submitted)

Roessingh, P. and E. Städler (1990): Influence of shape, size and colour of surrogate leaves on oviposition behaviour of *Delia radicum*. (This volume)

Schöni, R., E. Städler, J. A. A. Renwick and C. Radke (1987): Host and non-host plant chemicals influencing the oviposition behaviour of several herbivorous insects. In: V. Labeyrie, G. Fabres, and D. Lachaise (eds), *Proc. 6th Int. Symp. Insect-Plant Relationships, Pau 1986*. W. Junk, Dordrecht. 31-36.

Städler, E. (1978): Chemoreception of host plant chemicals by ovipositing females of *Delia (Hylemya) brassicae*. *Entomol. exp. appl. 24*, 711-720.

Städler, E. (1986): Oviposition and feeding stimuli in leaf surface waxes. In: B. E. Juniper and T. R. E. Southwood (eds), *Insects and the Plant Surface*. Edward Arnold, London. 105-121.

Städler, E. and H.-R. Buser (1984): Defense chemicals in leaf surface wax synergistically stimulate oviposition by a phytophagous insect. *Experientia 40*, 1157-1159.

Städler, E. and R. Schöni (1989): Oviposition behavior of the cabbage root fly, *Delia radicum* influenced by host plant extracts. *J. Insect Behav.* (in press)

Städler, E. and W. D. Seabrook (1974): Chemoreceptors on the proboscis of the female eastern spruce budworm: electrophysiological study. *Entomol. exp. appl. 18*, 153-160.

Tingle, F. C., R. R. Heath and E. R. Mitchell (1989): Flight responses of *Heliothis subflexa* (GN.) females (Lepidoptera: Noctuidae) to an attractant from groundcherry, *Physalis angulata* L. *J. Chem. Ecol. 15*, 221-231.

Traynier, R. M. M. and E. R. Hines (1987): Probes by aphids indicated by stain induced fluorescence in leaves. *Entomol. exp. appl. 45*, 198-201.

Visser, J. H., (1986): Host odor perception in phytophagous insects. *Ann. Rev. Entomol. 31*, 121-144.

Visser, J. H., (1988): Host-plant finding by insects: orientation, sensory input and search patterns. *J. Insect Physiol. 34*, 259-268.

Waladde, S. M., H. Kahoro, E. D. Kokwaro and M. Chimtawi (1985): Responses of *Chilo partellus* to material obtained from susceptible and resistant maize cultivars. Electrophysiology and behaviour. *Insect Sci. Appl. 6*, 341-347.

Waladde, S. M. and S. A. Ochieng (1990): Tarsi and ovipositor gustatory sensilla of *Maruca testulalis*: coding properties and behavioural responses. (This volume)

Wilson, D. D., R. F. Severson, K.-C. Son and S. J. Kays (1988): Oviposition stimulant in sweet potato periderm for the sweet potato weevil, *Cylas formicarius elegantulus* (Coleoptera: Curculionidae). *Environ. Entomol. 17*, 691-693.

Woodhead, S., C. Galeffi and G. B. Marini Betollo (1982): p-Hydroxybenzaldehyde as a major constituent of the epicuticular wax of seedling *Sorghum bicolor*. *Phytochem. 21*, 455-456.

Woodhead, S. and R. F. Chapman (1986): Insect behaviour and the chemistry of plant surface waxes. In: B. Juniper and T. R. E. Southwood (eds), *Insects and the Plant Surface*. Edward Arnold, London. 123-135.

Zobel, A. J. and S. A. Brown (1988): Determination of furanocoumarins on the leaf surface of *Ruta graveolens* with an improved extraction technique. *J. Nat. Prod. 51*, 941-946.

BEHAVIOUR

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INTERFERENCE WITH LEARNING IN PIPEVINE SWALLOWTAIL BUTTERFLIES: BEHAVIOURAL CONSTRAINT OR POSSIBLE ADAPTATION?

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ABSTRACT

A previous study found that ovipositing female pipevine swallowtail butterflies (*Battus philenor* L.) learned host leaf shape when *Aristolochia* host species were presented singly in outdoor enclosure arrays. When species were presented together, however, learning of one leaf shape interfered with learning of another. Here the effect of such interference on components of female fitness was examined under semi-field conditions. For two of three *Aristolochia* species presented singly, females found hosts at higher rates overall than when all host species were presented together. Host-finding rates increased with successive encounters when those two species were presented singly, but not when all three species were presented together. Improvement in host-finding with experience was associated only weakly with increases in oviposition rate. Rather, evidence suggested that females that find hosts at higher rates will be more selective about the plants on which they deposit eggs, placing more progeny on plants on which they are more likely to survive. The costs and benefits of learning (and interference with learning) are discussed by way of a model based on the Holling disc equation.

Key words: learning, memory, interference, functional response, specialization, phytopagous insects, oviposition, host selection, visual, search image, Papilionidae, Lepidoptera.

INTRODUCTION

Our intuition tells us that, like ourselves, insects can store and access only a limited amount of information about their environment. Such constraints are typically inferred from a tendency for new information to replace or to prevent access to previously stored information. Female *Colias* butterflies, for example, land progressively more often on host plants and less often on non-host plants over the course of an oviposition bout; they are apparently learning to recognize host plants before landing. If they then engage in nectar foraging, however, the frequency of landings on non-hosts (i.e., the frequency of errors) increases significantly at the beginning of the next oviposition bout (Stanton, 1984). Presumably as floral information is received, processed and stored during nectar foraging, information stored about host plants is lost or becomes difficult to access. A novel experience apparently interferes with recall of earlier experiences.

Similarly, learning to handle a second flower species interferes with a *Pieris* butterfly's recall of ability to handle the first species (Lewis, 1986), suggesting the

butterfly is unable to store or tap information about more than one flower species. An inability to remember simultaneously how to handle more than one flower type was implicit in Darwin's (1876) explanation for why bees often restrict their visits to flowers of one or several species while bypassing other species that are equally or even more rewarding. Memory is presumably under selective constraint.

Lewis (1986) noted a condition implicit in the notion of memory constraints, a condition that remains untested: the costs of interference with learning must exceed costs associated with specialization such as increased travel time between resource items. If this condition is not met, it might pay insects to switch readily among species, whether or not interference with learning reduces the effectiveness with which they exploit individual species. Rather than being a constraint, interference with learning might prevent an insect from specializing when such specialization was disadvantageous.

In this communication, I examine interference in a phytophagous insect that learns to search for host plants, the pipevine swallowtail butterfly (*Battus philenor* L.). Across their North American range, female pipevine swallowtail butterflies lay eggs exclusively on members of the genus *Aristolochia* in the Aristolochiaceae. Host-selection behaviour is relatively simple. Females searching for host plants land periodically on leaves. Once on the leaf, they taste the leaf surface with their foretarsi. If it is an *Aristolochia* leaf, they may or may not lay a small clutch; if it is not an *Aristolochia* leaf, they leave more or less immediately and resume searching.

While acceptance of a host plant for oviposition is governed by contact with leaf chemicals (Papaj, 1986a), finding the host in the first place is accomplished at least in part by orienting to the shape of a host's leaves. Ironically, it is the butterfly's mistakes, i.e., her landings on non-host leaves, that betray the importance of leaf shape in finding hosts. A given female tends to land periodically on non-host leaves similar in shape to those of the *Aristolochia* species for which she is searching (Rausher, 1978).

Responses to leaf shape are learned: experience with a particular host species induces a tendency to land on non-host leaves roughly similar in shape to that host (Papaj and Rausher, 1983; Papaj, 1986a). In an earlier study (Papaj, 1986b; Papaj, unpublished data), females presented with either the broad-leaved *A. macrophylla* or *A. reticulata* in enclosure arrays adopted a broad-leaf preference, while females presented with the narrow-leaved *A. serpentaria* adopted a narrow-leaf preference. Females presented with all three species at once, however, failed to adopt a clear preference for a particular shape, switching back and forth as they landed on hosts whose leaves differed in shape (Papaj, 1986b). Successive encounters with different host species apparently interfered with the butterfly's ability to learn a particular leaf shape.

Here I focus on the costs and benefits of such interference. Perhaps more directly than has been possible with respect to nectar-foraging, I characterize the components of fitness that might be influenced by learning with respect to oviposition-site selection and, hence, by interference with such learning. Finally, I show by way of a simple extension of the Holling disc equation how costs of specialization may occasionally

exceed costs of interference and thus how limits to memory may, contrary to intuition, sometimes improve reproductive success.

METHODS AND MATERIALS

Adults collected either as larvae in Tyler County, Texas, or as pupae at Thunder Ridge Lookout in Bedford County, Virginia, were used. Experiments were carried out in an outdoor enclosure at the Duke Zoology Field Station in Durham, North Carolina. Within the enclosure, fifty-five plants of each of eight non-host species were planted in the ground at random positions in a 60 m^2 rectangular grid. Four species had distinctly broad leaves and four had distinctly narrow leaves. In the interstices of the non-host array were distributed randomly 60 host plants of either *A. macrophylla*, *A. serpentaria*, *A. reticulata* or each of these host species in equal numbers. Additional details of arrays and plants are provided in Papaj (1986a,b).

Females marked individually with a unique number were released and allowed to search freely within an array. Each time a female entered the array, I counted her landings on broad-leaved and narrow-leaved non-host plants as well as any landings on hosts. Response to leaf shape was quantified in a Response Coefficient (RC) equal to the fraction of all non-host landings that were on broad leaves (Rausher, 1978). For perfect narrow-leaf searchers, RC=0; for perfect broad-leaf searchers, RC=1. Since the percentage of all landings that were on hosts (PCTHOST) is correlated strongly with actual host landing rate in the field (Spearman rank correlation, $r_s=0.78$, $p<0.0001$, $N=181$), PCTHOST was used to estimate host-landing rates in the enclosure. In order to examine how host-landing rates varied with RC, data for all oviposition bouts were pooled by female.

I also assessed how host-landing rates changed as females landed on more and more hosts. Because the number and duration of searching bouts and inter-bout intervals could not be controlled, standard repeated measures analyses were not used. Instead, searching bouts for all females were pooled within treatments. Differences among treatments in the relationship between arcsine-transformed values of PCTHOST within a bout and cumulative host landings prior to the beginning of that bout were examined with a weighted analysis of covariance adjusted for unbalanced data (SAS GLM; Freund and Littel, 1981; SAS Institute, 1982).

Changes in host-finding rates with experience could affect female reproductive success in at least two ways. First, females that find hosts at higher rates might lay eggs at higher rates. In the above enclosure experiment, I also recorded any ovipositions by females. Since the percentage of all landings (host and non-host) ending in oviposition (PCTOVO) was correlated strongly with actual oviposition rate (Spearman rank correlation, $r_s=0.97$, $p<0.0001$, $N=181$) in the field, PCTOVO was used to estimate oviposition rates in the enclosure. Changes in PCTOVO with cumulative host landings were analyzed as described for PCTHOST.

Alternatively, finding hosts at higher rates might permit females to be more selective about the plants on which eggs are deposited. In the following experiment,

Table 1 Percent of all landings that were on hosts (PCTHOST) by females presented with *Aristolochia macrophylla* (AM), *A. reticulata* (AR), *A. serpentaria* (AS) or all three species at once (ALL). Percentages followed by the same letter were not significantly different in a two-way G-test at an alpha level of 0.01.

Treatment	No. Females	No. Landings on		PCTHOST
		Hosts	Non-hosts	
AM	19	497	575	46.4 a
AR	34	585	952	38.1 b
AS	18	353	1014	25.8 c
ALL	18	674	1663	28.9 c

I asked whether females that find hosts at higher rates allocate proportionately more eggs to those plants on which their progeny are more likely to survive.

Low and high rates of host-finding were obtained by presenting females with *A. reticulata* at either of two densities, 20 or 60 plants. Within each density, plants varied in one characteristic, phenological age, known to affect both oviposition behavior and juvenile survival (Rausher and Papaj, 1983; Papaj and Rausher, 1987). Phenological age was manipulated by first cutting back plants to the root stock in order to synchronize leaf growth. After emergence of new leaves, half of the plants were cut back again. After several weeks, half of all plants bore mature foliage and half bore young foliage. If pruning induced any aberrant physiological response in the plants, it was not apparent in the behaviour of female butterflies: responses to plants prepared in this manner were identical in all respects to responses to plants in the field (Papaj and Rausher, 1987).

Thirty plants of each age were distributed randomly within the enclosure array. The low-density array consisted of the same 60 plants as the high-density array, except that 40 plants were always covered with a fiberglass-screen cage and rendered unavailable for contact and oviposition. Several times daily, cages were rotated to expose 20 different host plants. In this way, females in each treatment were eventually exposed to the same complement of host plants at the same spatial positions. Treatments were alternated over consecutive days.

As females searched within an array, I counted landings and ovipositions on host plants in each age category as well as landings on non-host plants. Since females avoid laying eggs on plants on which eggs are present (Rausher, 1979; Papaj, unpubl. enclosure data), plants were cleared periodically of eggs. The low frequency of ovipositions on phenologically old plants required that results for all females be pooled within density treatments and analyzed with non-parametric contingency tests.

RESULTS

Leaf-shape specialization and rate of host-finding. The percentage of landings that were on hosts (PCTHOST) in each of the four treatments is shown in Table 1. Overall, females presented with the broad-leaved *A. reticulata* or *A. macrophylla*

found hosts at higher rates than females presented with the narrow-leaved *A. serpentaria* or with all host species at once.

When females were presented with the broad-leaved *A. macrophylla* or *A. reticulata*, the association between PCTHOST and RC was positive (*A. macrophylla*: $r_s=0.28$, $p<0.10$, $N=19$; *A. reticulata*: Spearman rank correlation, $r_s=0.32$, $p<0.003$, $N=93$), suggesting that the stronger the preference for broad leaves the higher the rate at which broad-leaved hosts were found. When females were exposed to the narrow-leaved *A. serpentaria*, the association was negative ($r_s=-0.25$, $p<0.05$, $N=67$), suggesting that the stronger the preference for narrow leaves the higher the rate at which narrow-leaved hosts were found. No association between PCTHOST and RC was detected when females were presented with all host species at once ($r_s=0.03$, $p<0.80$, $N=18$).

PCTHOST within a trial increased with cumulative host landings for females presented with either *A. macrophylla* or *A. reticulata* (Fig. 1A and B), but changed less appreciably for females presented with *A. serpentaria* (Fig. 1C). PCTHOST changed little with cumulative host landings for females presented with all hosts species at once (Fig. 1A).

Analysis of covariance revealed a strong interaction between the change in PCTHOST with cumulative host landings and the host species to which females were exposed (Exposure x Cumulative Host Number effect, $F_{(3,581)}=28.27$, $p<0.0001$). Contrasts of single-host vs. mixed-host results and each pair of single-host results were made (SAS; Freund and Littell, 1981). The contrast between changes in PCTHOST in single-host arrays and those in the mixed-host array was highly signi-

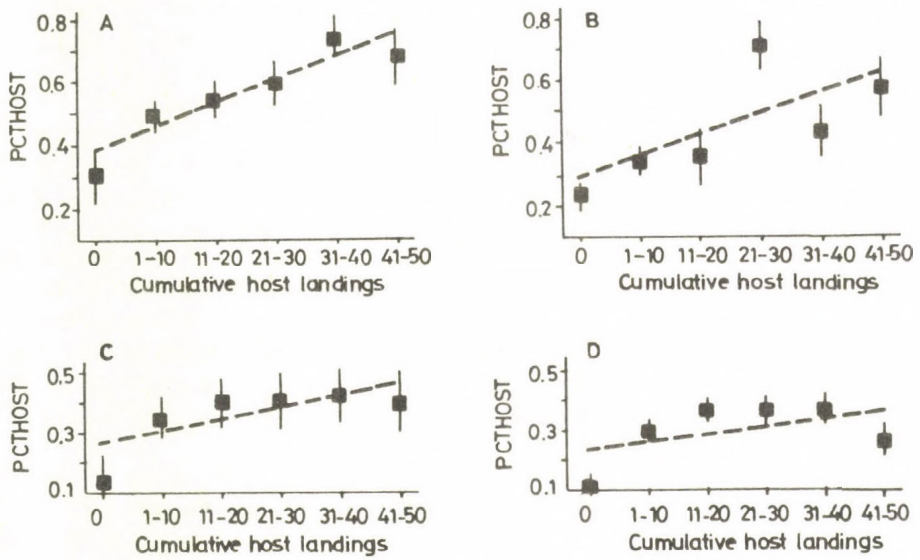

Fig. 1 Change in percent of all landings that were on hosts (PCTHOST) with cumulative host landings in an enclosure array containing (A) *A. macrophylla*, (B) *A. reticulata*, (C) *A. serpentaria* and (D) all host species. Shown are means and standard errors for bouts within a particular interval. Solid line is slope estimated in simple linear regression model.

Table 2 Percent landings that were on hosts (PCTHOST), percent of hosts on which females oviposited, and mean clutch size for phenologically old and young *A. reticulata* plants at two different densities in an enclosure array. Ovipositions on plants bearing previously laid eggs are not represented in data for percentages of hosts on which females oviposited.

Host Density	PCTHOST N	PCTHOST %	Plant Age	% Ovipositions N	% Ovipositions %	Mean Clutch Size (±1 s.e.)
			Old	32	43.8	7.68 (±1.40)
Low	1105	22.6				
			Young	72	55.6	6.12 (±0.86)
			Old	141	32.6	5.46 (±0.64)
High	1537	38.1				
			Young	116	62.1	6.58 (±0.50)

ficant ($F_{(1,581)}=21.94$; $p=0.0001$). In addition, changes in PCTHOST with experience in both *A. macrophylla* and *A. reticulata* treatments were significantly greater than those in the *A. serpentaria* treatment ($F_{(1,581)}=17.39$, $p<0.0001$ and $F_{(1,581)}=5.03$, $p<0.02$ respectively).

Leaf-shape specialization and rate of oviposition. For females presented with *A. reticulata*, the association between percentage of landings ending in oviposition (PCTOVO) and RC was positive and significant (Spearman rank correlation, $r_s=0.25$, $p<0.05$, $N=93$). For females presented with *A. macrophylla*, the association between PCTOVO and RC was positive, but not significantly so ($r_s=0.19$, $p>0.10$, $N=19$). For females presented with *A. serpentaria*, the association between PCTOVO and RC was negative, but not significantly so ($r_s=-0.12$, $p>0.3$, $N=67$). For females presented simultaneously with all three host species, the association between PCTOVO and RC was not significant ($r_s=-0.04$, $p>0.8$, $N=18$). In short, the rate at which females laid eggs was associated only weakly with leaf-shape specialization.

PCTOVO within a trial changed inconsistently with cumulative host landings for females presented with a single host species and not at all for females presented with all host species simultaneously. Analysis of covariance explained very little of the overall variance in the change in PCTOVO with cumulative host landings ($R^2=0.084$). Although the Exposure x Cumulative Host Number term ($F_{(3,581)}=8.95$, $p<0.0001$) revealed a significant interaction between change in PCTOVO with cumulative host landings and host treatment, contrasts failed to reveal significant differences among treatments. In particular, females presented with a single host species exhibited no greater change in PCTOVO with experience overall than did females presented with all host species simultaneously ($F_{(1,581)}=1.22$, $p>0.20.2$).

Host-finding and host selectivity. PCTHOST was substantially lower for females searching in the low-density *A. reticulata* array than for females searching in the high-density array (Table 2; G Test, $p<0.0001$). Females thus found hosts at higher rates at higher densities.

Females in the low-density array were less selective about plants on which they laid eggs than females in the high-density array (Table 2). At high host density, almost

74% of all eggs were laid on young plants, compared to just 53% at low density. This difference was not due to a difference in clutch size on young and old plants: the mean number of eggs laid per clutch on young vs. old plants did not depend on host density (Table 2; Two-Way ANOVA, Density x Plant Age effect, $F_{(1,168)}=2.57$, $p>0.1$).

Apparently, the difference in selectivity at low and high host density was due primarily to a difference in the tendency of females to lay eggs after landing on young vs. old plants. At high host density, the propensity of females to lay eggs after landing on young plants was significantly greater than on old plants (Table 2; G-test, $p<0.0001$). At low host density, the propensity of females to lay eggs after landing on young plants was not significantly different from that on old plants (Table 2; G-test, $p>0.1$).

DISCUSSION

Advantage of specialization on leaf shape. When host species were presented singly, females learned host leaf shape (Papaj, 1986a; Papaj, unpublished data). Current results suggest that such learning was associated with increases in the rate at which hosts were found. With respect to one host species, specialization on leaf shape was associated with increased rates of oviposition. For that species as well as others, increases in rates of host-finding may permit females to place more of their eggs on plants bearing young foliage on which progeny are more likely to survive (Table 2; Rausher 1983; Rausher and Papaj, 1983; Papaj and Rausher, 1987). Whether through effects on oviposition rate or juvenile survival, specialization on leaf shape should enhance a female's reproductive success, at least in monophagous populations.

One possible way in which orienting even crudely to host leaf shape increases host-finding rates in monophagous populations is simply stated: orientation to host leaf shape permits a somewhat myopic butterfly to ignore some non-hosts on which it might otherwise waste time in approaching and examining. This argument can be stated formally in a simple model.

Suppose that the rate at which hosts are found by a female (R_H) increases with host density to an asymptote as shown in Fig. 2A. This pattern is typical of ovipositing *B. philenor* in the field (Rausher, 1983) and can be described by the well-known disc equation (Holling, 1959):

$$R_H = \frac{aD_H}{1+aD_Hh_H}$$

in which D_H is the density of the host, h_H is the handling time required to examine a host (i.e., the handling time) and a is a constant which describes the "rate of successful search". R_H levels off with increasing host density because as hosts are found at higher rates, an ever greater proportion of time is spent examining host plants and an ever smaller proportion is available for searching. R_H eventually reaches an asymptote equal to the reciprocal of the handling time, h_H.

To quantify how wasting time on non-host leaves reduces the rate at which females find hosts, I used a modification of the disc equation for the case in which a predator searches for two prey species. In this case, one of the 'prey' -- i.e., the non-host -- has no value and a correspondingly short handling time (h_{NH}). The effect of time wasted on non-host plants on R_H is illustrated by the solid lines in Fig. 2A. Even if approaching, examining and leaving a non-host leaf takes just two seconds, R_H is reduced markedly. The more non-hosts occur in the habitat, the more R_H is reduced.

The advantage of specializing on host leaf shape can be demonstrated with the modified disc equation, by reducing the density of non-hosts by the fraction whose

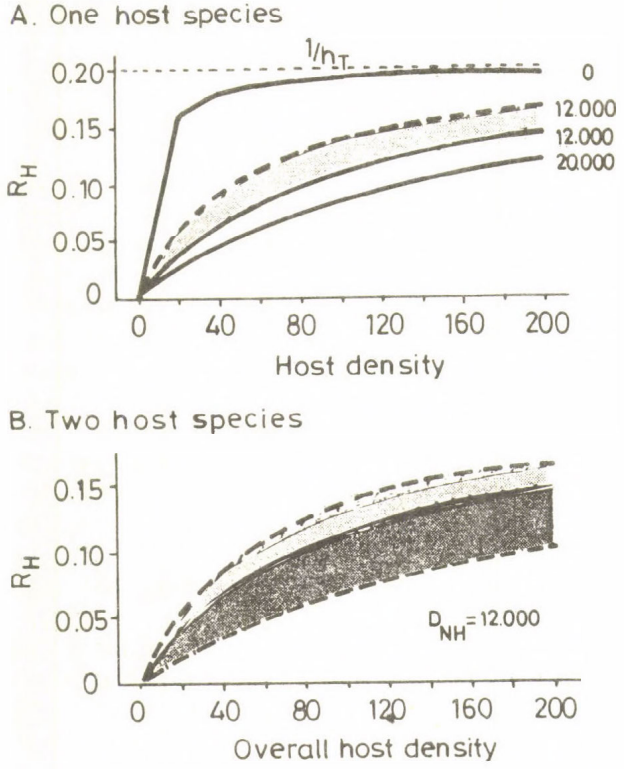

Fig. 2 A. The effect of non-host density and specialization on leaf shape on the rate at which hosts are found (R_H) in single-host assemblages. Parameters were assigned the following values: h_H=5 min, h_{NH}=2 sec and a=0.4. To right of each curve is indicated non-host density. Solid lines indicates leaf-shape generalist. Dashed line indicates specialist on leaf shape of host. Light grey area shows advantage to specialist on leaf shape. B. The effect of specialization on leaf shape on R_H in a two-host assemblage where host species differ in leaf shape. Host A is assumed to outnumber Host B by a margin of 4:1. Solid line indicates leaf-shape generalist. Upper dashed line indicates specialist on leaf shape of Host A; lower dashed line indicates specialist on leaf shape of Host B. Light grey area shows advantage to specialist on Host A; dark grey area shows disadvantage to specialist on Host B. Parameters as in A.

leaf shape differs from that of the host. Suppose that the butterfly can resolve only two categories of shape, broad and narrow. Suppose that half of the non-hosts in the habitat are broad-leaved and half are narrow-leaved. As shown in Fig. 2A, a butterfly that orients to the shape of its host's leaves and, by doing so, ignores half of all non-host plants finds hosts faster at a given overall host density than one that does not. So long as there exist non-hosts with leaves different in shape from those of the host, it pays to orient even crudely to shape. The exact payoff depends on the density of both host and non-host plants as well as on the proportion of non-hosts whose leaves resemble those of the host.

Disadvantage of interference. When host species were presented together in the enclosure, females failed to adopt a preference for a particular host leaf shape, switching back and forth as they alighted on hosts whose leaves differed in shape (Papaj, 1986b). Successive encounters with different host species apparently interfered with the butterfly's ability to learn a particular leaf shape. The model presented above suggests that, by preventing specialization on host leaf shape and thus increasing time wasted on non-host leaves, interference with learning should reduce the rate at which hosts are found. The fact that females found hosts at significantly lower rates when all host species were presented at once in the enclosure than when two of three host species were presented singly at the same overall density (Table 1) is consistent with this prediction. By diminishing host-finding rate, interference with learning presumably reduces a female's reproductive success in the same way as learning enhances it, by altering either her rate of oviposition or the survival of her progeny.

Note that this cost of interference with learning has nothing to do with time wasted in learning and relearning, as described by Lewis (1986) with respect to nectar-foraging butterflies and flower-handling. The cost put forward here is really the cost of being a leaf-shape generalist and is borne regardless of level of experience. Interference-prone females may suffer an additional cost in learning and relearning leaf shape, analogous to that assessed by Lewis (1986).

Advantage of interference. Insects prone to interference should clearly avoid host-diverse assemblages if they have the opportunity to search for a single host at the same overall density. If that is not an option, however, insects confused by the diversity of host species may sometimes do better than those that are faithful to just one host species.

Consider a habitat containing two host species, one broad-leaved and one narrow-leaved, in which half of the non-hosts are broad-leaved and half are narrow-leaved. Suppose R_H for a leaf-shape generalist that lands indiscriminately with respect to leaf shape is the same in the two-host habitat as in habitats containing only one host species at the same overall density (Fig. 2). R_H for a specialist on the shape of one host's leaves depends on the density of that host relative to the other. If one host species is sufficiently more abundant than the other, a specialist on the common species finds hosts faster than the generalist (Fig. 2B): the decrease in R_H due to ignoring the rare species is apparently offset by the increase due to avoiding half of the non-hosts. A specialist on the rare species, however, does much worse

than the generalist (Fig. 2B): the decrease in R_H due to ignoring the common host exceeds greatly the increase due to avoiding half of the non-hosts.

Interestingly, when host species are equally abundant, leaf-shape generalists always hold the advantage, regardless of the density of hosts or non-hosts (Fig. 3A). Under such circumstances, it should always benefit the butterfly if chance encounters with the second host species interfered with any preference acquired upon encounter with the first species.

Just as interestingly, it does not necessarily pay to specialize whenever one host is more abundant than the other. Whether specialists are favoured over generalists depends on the value of the constant *a*. If *a* is small enough, the generalist is at an advantage even if one host species outnumbers another by a margin of 4:1 (Fig. 3B). This may explain why butterflies were leaf-shape generalists in the mixed-host array.

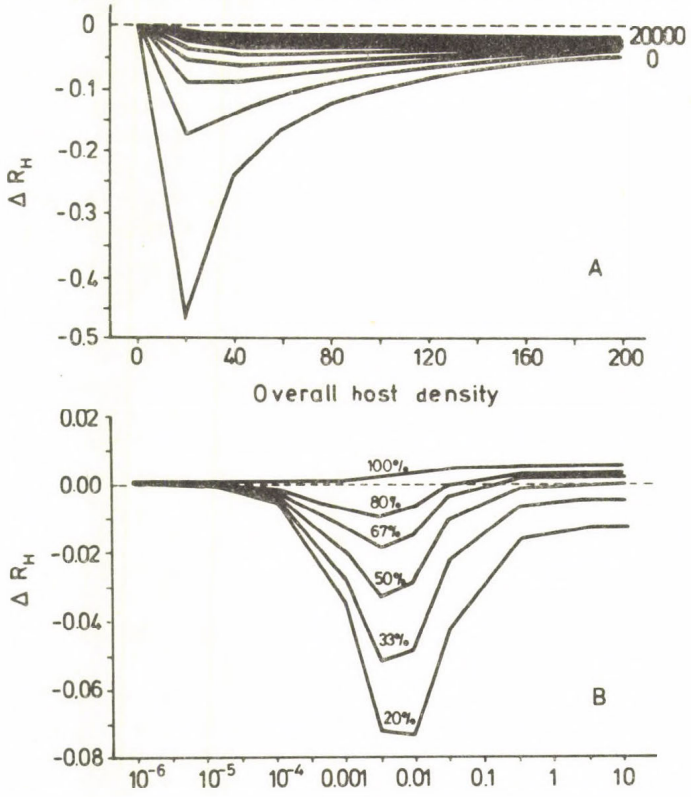

Fig. 3 A. Difference in rate of host-finding (ΔR_H) between leaf-shape specialist and generalist as a function of overall host density and non-host density. Non-host density was varied between 0 and 20,000 in increments of 2,000. Dashed line indicates line of no advantage to either specialist or generalist. Parameters as in Fig. 2.-B. ΔR_H in a two-host assemblage as a function of *a*, the rate of successful search. Percentages refer to percentage of all hosts that are of type A. Dashed line indicates line of no advantage to specialist or generalist. Overall host and nonhost densities from enclosure experiment were used in calculations. Other parameters as in Fig. 2.

Despite the overall reduction in rate of host-finding and even though broad-leaved hosts outnumbered narrow-leaved hosts by a margin of 2:1, generalists would be favoured over broad-leaf specialists so long as a did not exceed 0.3 (Fig. 3B). In short, there are clearly circumstances when interference with learning, rather than being a constraint which cannot be altered by natural selection, is functional and potentially adaptive.

A feast of assumptions. The shortcomings of the Holling disc equation are well known. It assumes that host density remains constant as the insect is foraging. Since butterflies avoid laying eggs on plants that already contain eggs (Rausher, 1979), this assumption is unrealistic. In effect, host density declines as the butterfly deposits clutches (except perhaps in the enclosure where eggs were removed regularly). The disc equation also assumes that a is constant when, in fact, it usually declines with host density (cf. Rausher 1983). It also ignores effects of satiation or, in this case, of egg depletion. The modified disc equation assumes that host and non-host plants are not patchy in distribution, but are intermingled throughout the habitat. This assumption is unrealistic in the field (though probably not in the enclosure where plants were distributed randomly).

None of these general criticisms of the Holling disc equation should jeopardize our conclusions with respect to the advantages and disadvantages of specialization on leaf shape. Other assumptions specific to the present model might, however. The model assumes, for example, that a is not affected by experience. If butterflies formed search images (*sensu* Guilford and Dawkins, 1987) in which experience increased the probability of detecting a cryptic host once encountered, a would increase with experience. This would increase the advantage to specializing on leaf shape and restrict the conditions under which interference with learning is useful to the insect. This caveat notwithstanding, my model is perhaps most useful precisely because it emphasizes changes in the probability of attacking 'false hosts' given an encounter rather than changes in the probability of attacking the actual host given an encounter. Most previous models of foraging for cryptic prey emphasize the latter probability (see Guilford and Dawkins, 1987).

Finally, the model puts emphasis entirely on how landing on non-hosts affects the rate at which hosts are found. Reducing the time spent on non-host leaves might affect fitness in other ways, e.g. by altering a female's risk to predation. Avoiding landings on non-host leaves may reduce attack by ground predators such as lizards and spiders, increase attack by aerial predators such as dragonflies and birds, or both. The payoff to specialization on leaf shape may depend on the balance of these risks.

Where's the constraint? Regardless of these and other assumptions, the model is useful if only because it challenges the intuitively appealing notion that the susceptibility of memory to interference by novel events reflects a constraint on directional selection. Given what appears to be a limitation in the ability of butterflies to resolve leaf shape, absent-mindedness might well have selective value. With respect to properties of memory, intuition may be an exceptionally misleading guide. The careful reader will take note, though. In dismissing one constraint, we accept another. The implication that the visual acuity of insects is under selective constraint appeals

once more to intuition, perhaps appealing more to this spectacled biologist than to others.

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REFERENCES

Darwin, C. (1876): *On the Effects of Cross and Self Fertilisation in the Vegetable Kingdom.* John Murray, London.

Freund, R. J. and R. C. Littell (1981): *SAS for Linear Models: A Guide to the ANOVA and GLM Procedures.* SAS Institute, Cary, North Carolina

Guilford, T. and M. S. Dawkins (1987): Search images not proven: a reappraisal of the evidence. *Anim. Behav. 35*, 1838-1845.

Holling, C. S. (1959): The components of predation as revealed by a study of small mammal predation of the European pine sawfly. *Can. Entomol. 91*, 293-320.

Lewis, A. C. (1986): Memory constraints and flower choice in *Pieris rapae. Science 232*, 863-865.

Papaj, D. R. (1986a): Conditioning of leaf-shape discrimination by chemical cues in the butterfly, *Battus philenor. Anim. Behav. 34*, 1281-1288.

Papaj, D. R. (1986b): Interpopulation differences in host preference and the evolution of learning in the butterfly *Battus philenor. Evolution 40*, 518-530.

Papaj, D. R. and M. D. Rauscher (1983): Individual variation in host location by phytophagous insects. In: S. Ahmad (ed), *Herbivorous Insects: Host-seeking Behavior and Mechanisms.* Academic Press, New York. 77-124.

Papaj, D. R. and M. D. Rausher (1987): Components of conspecific host plant discrimination by *Battus philenor* (Papilionidae). *Ecology 68*, 245-253.

Rausher, M. D. (1978): Search image for leaf shape in a butterfly. *Science 200*, 1071-1073.

Rausher, M. D. (1979): Egg recognition: its advantage to a butterfly. *Anim. Behav. 27*, 1034-1040.

Rausher, M. D. (1983): Alteration of oviposition behavior by *Battus philenor* butterflies in response to variation in host-plant density. *Ecology 64*, 1028-1034.

Rausher, M. D. and D. R. Papaj (1983): Demographic consequences of conspecific discrimination by *Battus philenor* butterflies. *Ecology 64*, 1402-1410.
Stanton, M. L. (1984): Short-term learning and the searching accuracy of egg-laying butterflies. *Anim. Behav. 32*, 33-40.

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ORIENTATION TO HOST PLANT AND CONSPECIFICS BY THE BOLL WEEVIL, *ANTHONOMUS GRANDIS* BOH. (COLEOPTERA: CURCULIONIDAE) ELECTROPHYSIOLOGICAL AND BEHAVIORAL CORRELATIONS

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ABSTRACT

Single cell recordings from antennal olfactory cells in the boll weevil revealed receptors for three aggregation pheromone components and six host plant odors with no overlap among receptors for each class of odorant. While receptors for pheromones and host odors were equally distributed in males, a higher percentage of cells in females responded to the aggregation pheromone. Distribution of the specific plant odor receptors were similar for both males and females. Cells responsive to β-caryophyllene, green leaf volatiles, and *trans*-β-ocimene, accounted for 80% of the receptors recorded in both sexes. Behavioral tests showed that while these latter three odorants were only slightly attractive alone, when in combination with the aggregation pheromone they enhanced its attractiveness nearly twofold.

Key words: single cell recording, aggregation pheromone, distribution of receptors, β-caryophyllene, green leaf volatiles, trans-β-ocimene, attraction.

INTRODUCTION

The relationship of insects to their host plants and conspecifics is interesting since both pheromones and host odors (kairomones) interact in the olfactory-mediated behavior of the insect. The boll weevil is narrowly oligophagous and feeds only on its host plant, cotton (*Gossypium hirsutum* L.), and closely related Malvaceae (Cross et al., 1975). Upon feeding on their host plant, male boll weevils produce an aggregation pheromone which is released in their frass (Tumlinson et al., 1969).

The role of plant volatiles in the behavior of the boll weevil has been a subject of debate. Several investigators reported that the boll weevil was attracted to its host plant by volatiles emanating from it, while others considered that the boll weevil located its host plant by random flights (see references in Dickens, 1984). Other investigators found various compounds to be attractive to boll weevils in laboratory bioassays (see references in Hedin et al., 1973). Recently, a mixture of monoterpenes emitted by blooming cotton was found to be attractive in laboratory bioassays, and enhanced responses of the boll weevil to its aggregation pheromone in field tests (Chang, 1986). Most recently green leaf volatiles were found to enhance response of the boll weevil to its aggregation pheromone (Dickens, 1989a).

The relationship of the boll weevil to the cotton plant has been largely investigated by chemists who have identified over 250 compounds from the plant, and by entomologists who have monitored activity of selected chemicals in traps. I report herein results of single cell investigations of the olfactory receptor system of the boll weevil, and initial experiments to determine effects of key compounds on boll weevil behavior.

MATERIALS AND METHODS

Insects. Adult *A. grandis* were obtained from a small laboratory colony annually infused with feral insects. Upon emergence, insects were sexed and fed cotton squares, when available, or an artificial diet (Lindig, 1979). Insects were not used until substantial olfactory receptor maturation at four days postemergence (Dickens and Moorman, 1987). For electrophysiological studies, groups of five insects of the same sex were maintained on moist filter paper (Whatman™ #1) in Petri dishes until use at four to eight days of age. For behavioral experiments, groups of fifty insects of the same sex were held in paper cartons (ca. 0.5 liter) with screen tops. Insects used in the behavioral experiments were six to thirteen days of age. All insects were held in incubators at 26°C under a photoregime of 16 hours of light (ca. 700 lux) and 8 hours of darkness.

Chemicals. Odorous stimuli were selected based on their presence in the cotton plant (Hedin et al., 1973) and their activity in an earlier electroantennogram (EAG) study (Dickens, 1984). Compounds tested, their purity in parentheses, included: hexan-1-ol (99), *trans*-2-hexen-1-ol (97), *trans*-2-hexenal (99), (-)-α-pinene (98), (-)-β-pinene (80-90), myrcene (85), (-)-limonene (85), *trans*-β-ocimene (60), (-)-β-citronellan (95), nerol (65), geraniol (90), linalool (99), α-bisabolol (92), β-bisabolol (83), β-caryophyllene (90), and benzaldehyde (99). A mixture of monoterpenes [1R(+)-α-pinene, 1S(-)-β-pinene, β-myrcene, R-(+)- d-limonene, and *trans*-β-ocimene in a ratio of 53:11:16:4:16] isolated from blooming cotton and found to enhance responses of boll weevils to their aggregation pheromone, grandlure, was also tested (Chang, 1986; J. H. Benedict, personal communication). Individual components of the monoterpene blend were also tested. Grandlure, a mixture of compounds I:II:III+IV in the ratio 3:4:3 was also tested. All grandlure components were 90% chemically pure. Serial dilutions were prepared in hexane.

Single cell recordings. Single cell recording techniques are described in detail elsewhere (Dickens and Mori, 1989). In brief, microelectrodes were constructed from 50.8 μm diameter tungsten wire electrolytically sharpened to a tip of 1-2 μm. The recording electrode was positioned near either the proximal edge of one of the three sensory bands encircling the club or the base of an individual sensillum just distal to the sensory band region. The ground electrode was inserted in the distal end of the scape. Action potentials were amplified by a Grass P-15 preamplifier, stored on cassette tapes using a Teac R51-D data recorder, and afterwards photographed from the oscilloscope with a Tektronix C-5C camera.

Experimental protocol. Upon obtaining a single cell preparation, the first two stimuli tested were volatiles emanating from 1 μg of grandlure, followed by volatiles emanating from a crushed cotton square. These two stimuli served to determine whether the cell was activated by the aggregation pheromone of the boll weevil and/or volatiles emanating from its host and food source.

If the cell were activated by volatiles from the crushed cotton square or was not activated by the pheromone, then presentation of 1 μg doses of other selected odorants followed. Odorous stimuli were delivered as 1 μl aliquots placed on filter paper (8 mm x 18 mm) inserted into glass cartridges (80 mm x 5 mm ID) oriented toward the preparation from a distance of 1 cm. Molecules evaporating from the filter paper were carried over the preparation by hydrocarbon-free air which also had been filtered and dried. Stimulus duration was 0.5 sec with an air flow of ca. 1 liter/min. Two or three minutes were allowed between each stimulation.

Behavioral experiments. Two field experiments involving an array of four traps were performed. One trap was placed at each corner of a 20 m square. The two treatments being tested simultaneously in this competitive arrangement were placed alternately in the traps, i. e. the two traps baited with the same treatment were located diagonally from each other. Odorants diluted in hexane were released from 2.2 ml vials as 10 μg/μl dilutions using glass capillaries (Dickens and Mori, 1989). In one test, two traps were baited with 1 mg of a 1:1:1 mixture of β-caryophyllene, *trans*-2-hexen-1-ol, and *trans*-β-ocimene. The other two traps were left unbaited. In a second test, one vial placed in each trap contained (±)-I (0.4 mg); a second vial contained II (0.3 mg) and III+IV (0.1 mg). The ratio of the pheromone components used in these studies [I(4):II(3):III+IV(1)] approximated that of the natural pheromone as identified from the frass produced by male boll weevils (Tumlinson et al., 1969). Two of the traps also contained a third vial with 1 mg of a 1:1:1 mixture of β-caryophyllene, *trans*-2-hexen-1-ol, and *trans*-β-ocimene. Following transport to the field in a cooler, the teflon-lined screw caps were removed from the vials and both caps and vials were placed in the top of an aluminum film container (3 cm diam. x 4.3 cm height) that had 5 holes (0.23 mm) drilled near its outer edge. The bottom of the can was then screwed onto the top and the inverted film container was placed in the trap below the screen funnel.

For each replicate, 100 insects (50 males + 50 females) were released along a 20 m line parallel to each side of the square at a distance of 20 m (400 insects total) at 1400 h. Traps were then immediately baited. In each test, traps were checked and insects removed at 1530 h and 1700 h on the day of release, and before 0900 h the next day (the time at which the test was terminated). Both tests were replicated three times.

RESULTS

Single cell recordings. Receptors for both pheromones and host plant odors were housed within sensilla in each of the three sensory band regions on the antennae of

Table 1 Example of responses of receptor cells for plant odors in the boll weevil, *Anthonomus grandis*.

Odorant	Cell	Impulses/initial 400 msec					
		# 1	# 2	# 3	# 4	# 5	# 6
grandlure		4	2	3	3	2	3
crushed cotton square		23	2	1	13	5	4
trans-2-hexen-1-ol		1	2	6	68	8	2
monoterpene mixture		3	19	7	7	7	4
trans-β-ocimene		2	20	-	3	-	8
linalool		6	5	6	8	6	60
β-bisabolol		5	13	3	6	13	4
β-caryophyllene		35	8	5	4	5	1
benzaldehyde		2	10	24	6	-	12
spontaneous activity		3	5	3	7	5	2

both male and female *A. grandis* (Dickens, 1989b). Due to the fact that the sensilla within the sensory bands are packed closely together, it was impossible to discern individual hairs. However one might assume that accessible hairs were recorded at random in both males and females with the result being that the number of cells recorded for a given odorant represented a fair assessment of the insect's olfactory endowment for its detection. While receptors for pheromones and plant odors in males were equally distributed, a higher percentage of cells recorded in females were

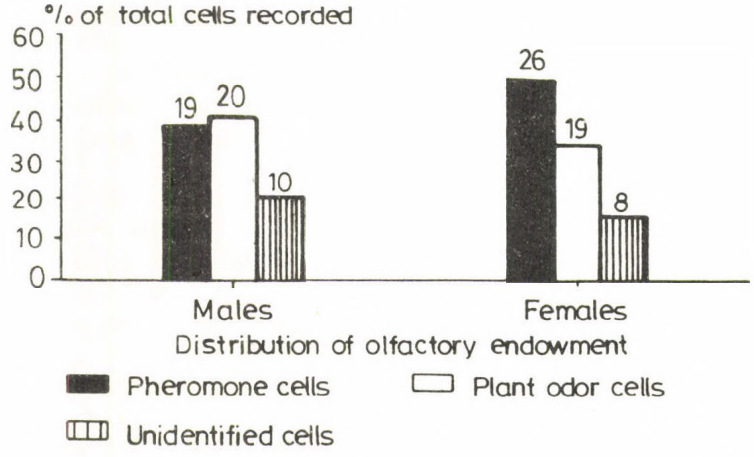

Fig. 1 Endowment of receptor cells for pheromones and host odors in male and female *Anthonomus grandis*. Numbers in parentheses indicate actual numbers of receptor cells recorded.

responsive to the aggregation pheromone than to plant odors (Fig. 1). It should be pointed out that no overlap was noted in responses of the pheromone and plant odor receptors.

Receptors for plant odors responded to one of six odorants with little or no overlap among most receptors (Table 1). Cells responsive to green leaf volatiles and linalool were strongly activated by volatiles emanating from 1 μg of their key odorant. Receptors for β-caryophyllene, and benzaldehyde were moderately activated by this dose. These four receptor types showed the greatest specificity for their key compounds at this dose. Cells responsive to *trans*-β-ocimene and β-bisabolol were only mildly activated at this dose and showed the least specificity.

Distribution of the specific plant odor receptors were similar for both males and females (Fig. 2). β-caryophyllene receptors were the most frequently encountered and accounted for more than 40% of identified receptors in both sexes. Receptors for green leaf volatiles were the next most frequently recorded receptor type with a slightly higher percentage of these receptors recorded in males (30.1%) than in females (20%). Cells responsive to β-caryophyllene, green leaf volatiles, and *trans*-β-ocimene, accounted for 80% of the receptors for plant odors recorded in both sexes. Each of the other receptor types was encountered relatively infrequently.

Behavioral tests. Results of the initial field experiment showed that the combination of β-caryophyllene, *trans*-2-hexen-1-ol, and *trans*-β-ocimene at the dose tested

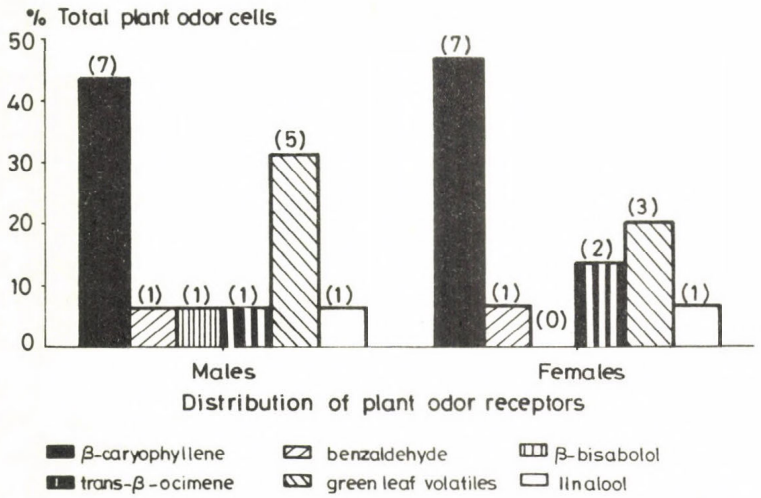

Fig. 2 Distribution of plant odor receptor cells in male and female *Anthonomus grandis*. Numbers in parentheses indicate actual numbers of receptor cells recorded.

Table 2 Mean number of boll weevils captured in three replicates of competitive field tests

Treatments	Mean trap capture	Sex ratio (M:F)
Unbaited trap	0	
β-caryophyllene, *trans*-2-hexen-1-ol, *trans*-β-ocimene (1 mg)	1.3	3.0:1
grandlure (0.8 mg)	17.7	1.3:1
grandlure (0.8 mg) + β-caryophyllene, *trans*-2-hexen-1-ol, *trans*-β-ocimene (1 mg)	33.0*	1.5:1

*$P<0.05$, paired t-test

attracted only a few weevils (Table 2). It is interesting that three of the four weevils captured were males, the sex responsible for pheromone production.

The second field experiment showed that the relatively unattractive combination of β-caryophyllene, *trans*-2-hexen-1-ol, and *trans*-β-ocimene when combined with grandlure enhanced the attractiveness of grandlure nearly two-fold when in competition with grandlure alone.

DISCUSSION

The investment in olfactory receptor cells for both pheromones and host plant odors by male and female boll weevils clearly demonstrates the importance of each class of odorant in boll weevil behavior. The fact that females have a somewhat larger investment in receptors for the male-produced pheromone is in accordance with the need for the female to mate. Both males and females maintain a large investment in host plant receptors, since males must locate their host plant for feeding and pheromone production, and females must sense their host plant for feeding and oviposition.

The lack of overlap in response spectra of pheromone and plant odor receptors indicate that these two types of information pass along separate channels to the brain as has been found for other Coleoptera (Mustaparta et al., 1979; Dickens et al., 1984). The specialization of receptor cells for several host plant odors with diverse chemical structures and common occurrence in plants is surprising. The paucity of specialized receptors for β-bisabolol, a compound reported only from cotton and closely related Malvaceae (Thompson et al., 1971), is difficult to explain. The fact that each key host plant odor is widely distributed among plants indicates that the boll weevil uses an odorous blend to detect its host plant as proposed for another coleopteran (Visser and Avé, 1978).

Our behavioral tests demonstrate that a minimal blend of the three compounds which activate 80% of the plant odor receptor cells in both sexes may be slightly attractive in the field (Table 2). Once the host plant is located by a male, feeding and pheromone production follow (Tumlinson et al., 1969). The three compounds, which were slightly atttractive before, now enhance response of both sexes to the pheromone nearly twofold (Table 2).

In conclusion, host plant location by a narrowly oligophagous insect such as the boll weevil involves a wide range of chemical structures detected by relatively specialized receptors. Once the insect locates its host plant and pheromone production begins, the ensuing semiochemical blend is an enhanced attractant for other receiving insects.

REFERENCES

Chang, J. F. F. (1986): Influence of cotton cultivars on boll weevil (Coleoptera: Curculionidae) behavior and pheromone production. *Ph. D. Thesis. Texas A&M University.*

Cross, W. H., M. J. Lukefahr, P. A. Fryxell and H. R. Burke (1975): Host plants of the boll weevil. *Environ. Entomol. 5*, 565-571.

Dickens, J. C. (1984): Olfaction in the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae): Electroantennogram studies. *J. Chem. Ecol. 10*, 1759-1785.

Dickens, J. C. (1989a): Green leaf volatiles enhance aggregation pheromone of boll weevil, *Anthonomus grandis. Entomol. exp. appl.* (in press).

Dickens, J. C. (1989b): Specialized receptor cells for pheromones and host plant odors in the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae). (in review)

Dickens, J. C. and E. E. Moorman (1987): Role of host plant and sensory environment in maturation of olfactory receptor system of the boll weevil, *Anthonomus grandis. Neurosci. Abstr. 13*, 82.

Dickens, J. C. and K. Mori (1989): Receptor chirality and behavioral specificity of the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae) for its pheromone, (+)-grandisol. *J. Chem. Ecol. 15*, 517-528.

Dickens, J. C., T. L. Payne, L. C. Ryker and J. A. Rudinsky (1984): Single cell responses of the Douglas-fir beetle, *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae) to pheromones and host odors. *J. Chem. Ecol. 10*, 583-600.

Hedin, P. A., A. C. Thompson and R. C. Gueldner (1973): The boll weevil-cotton plant complex. *Toxicol. Environ. Chem. Rev. 1*,291-351.

Lindig, O. H. (1979): A replacement for cottonseed meal and meats in boll weevil diets. *J. Econ. Entomol. 72*, 291-292.

Mustaparta, H., M. E. Angst and G. N. Lanier (1979): Specialization of olfactory cells to insect- and host-produced volatiles in the bark beetle *Ips pini. J. Chem. Ecol. 5*, 109-123.

Thompson, A. C., B. W. Hanny, P. A. Hedin and R. C. Gueldner (1971): Phytochemical studies in the family Malvaceae. I. Comparison of essential oils of six species by gas-liquid chromatography. *Amer. J. Bot. 58*, 803-807.

Tumlinson, J. H., D. D. Hardee, R. C. Gueldner, A. C. Thompson, P. A. Hedin and J. P. Minyard (1969): Sex pheromones produced by male boll weevils: Isolation, identification, and synthesis. *Science 166*, 1010-1012.

Visser, J. H. and D. A. Avé (1978): General green leaf volatiles in the olfactory orientation of the Colorado beetle, *Leptinotarsa decemlineata. Entomol. exp. appl. 24*, 538-549.

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ELECTROPHYSIOLOGICAL RESPONSES OF TARSAL CHEMORECEPTORS OF *HELIOTHIS VIRESCENS* (F.) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The tobacco budworm moth, *Heliothis virescens*, has non-porous, trichoid sensilla and 2 kinds of uniporous, contact chemosensilla on the ventral surface of its tarsi. Both types of chemosensilla respond electrophysiologically to sucrose, NaCl, KCl, and plant extracts. The sensilla exhibit differences in spike frequency, amplitude, and shape in response to various compounds.

Key words: *Heliothis virescens*, electrophysiology, tarsi, contact chemosensilla, mechanosensilla.

INTRODUCTION

Adults of *Heliothis virescens*, the tobacco budworm (TBW), extend their proboscis upon stimulation of tarsi or antennae with sugars (Ramaswamy, 1987). The ovipositor also has sugar sensitive sensilla (Ramaswamy and Hanson, unpublished). Gravid females discriminate between host and nonhost plants for oviposition via chemosensory, mechanosensory, and visual information from tarsi, ovipositor, and compound eyes (Ramaswamy et al., 1987; Ramaswamy, 1988; Navasero and Ramaswamy, 1989). Although a few studies have characterized the electrophysiological properties of contact chemoreceptors (e.g., Waladde et al., 1985), the physiological basis of host location by moths is poorly understood. This is a preliminary report on the morphology and electrophysiological responses to sugars, salts and plant extracts, of tarsal sensilla of adult TBW involved in host discrimination.

PROCEDURES

TBW larvae were raised on a wheat germ diet. Adults were housed in an environmental chamber at 14:10 L:D, 26°C, 75% RH and fed distilled water. Moths were used in the studies within 24 h of emergence. Molar solutions of sugars and salts (Sigma, St. Louis, MO) used in tests were made in glass distilled water.

Terminal leaves and flowers from 2 hosts of TBW (tobacco, cotton) and a non-host (ground cherry; *Physalis angulata* L.) were excised, weighed, and extracted in meth-

anol (1 ml/g fresh tissue weight) for 30 sec. Extracts were concentrated under N_2 to 10% of their original volume, mixed with an equal part of 0.1 M NaCl, and filled into the stimulating/recording electrodes.

The tip-recording technique (Frazier and Hanson, 1986) was used to measure electrophysiological responses of chemoreceptors to sugars, salts and plant extracts. A moth, with wings cut at the base, was restrained ventral side up with adhesive tape in a cavity excavated in wax in a small glass petri dish. Its legs were restrained with adhesive tape to expose only the ventral tarsal surface. The indifferent electrode was a glass pipet, ca. 0.1 mm diam., containing 0.1 M NaCl and inserted into the thorax of the moth. A similar glass pipet containing the stimulus in 0.05 M NaCl was slipped over the sensillum tip. Signals were amplified by a baseline restoring preamplifier and a bandpass amplifier and displayed on an oscilloscope. Signals were recorded on magnetic tape for subsequent analysis and plotting (Hanson et al., 1986). Responses of sensilla on the last tarsomere of all 3 pairs of tarsi were measured.



Fig. 1 Ventral surface of right hind tarsomere 5 of female *Heliothis virescens*. –▷ = Type A trichoid nonporous sensilla; ▷ = Type B contact chemosensilla; –► = Type C contact chemosensilla.

RESULTS AND DISCUSSION

The ventral tarsal surface of TBW has 3 kinds of sensilla (Fig. 1). The trichoid, non-porous (Type A; open arrow in Fig. 1) are robust, of variable length (length range = 150-350 μ; base range = 10-45 μ), and have prominent longitudinal ridges. They neither take up reduced silver nor respond to stimulation by the tip recording method, suggesting they lack pores at the tip and may be mechanosensory. The trichoid, uniporous B sensilla (length = 71.8±9.4 μ; base = 4.8±0.6 μ; arrowhead in Fig. 1) are curved, less robust and lack ridges. They are more predominant at lateral and distal margins of the tarsomere. The more robust, trichoid, uniporous C sensilla (length = 42.1±3.9 μ; base = 8.8±1.5 μ; closed arrow in Fig. 1) have sharply recurved tips, lack ridges, and are always arranged in a row off midline. The latter 2 types have single pores at the tip, take up reduced silver into the lumen and respond to stimulation with chemicals in solution.

The B and C sensilla respond similarly to sucrose, NaCl, KCl, or plant extracts. Three cells respond to NaCl with different spike amplitudes and narrow negative phase (Fig. 2a). The 3 salt cells do not exhibit dose-dependent responses to NaCl (Fig. 2c), although spike frequency in the large cell of ca. 20% of the preparations increases with increasing NaCl concentrations. The cells adapt to 0.5 M NaCl but not to 0.1 M or lower concentrations (Fig. 2d). The salt spike increases in frequency and changes in shape over time, and spike height increases with concentration. Two cells, and perhaps a third, respond to KCl; possibly they are the same as those responding to NaCl but with a different spike shape (Fig. 2b). The ability to perceive salts may allow location of supplemental sources of salts, especially Na^+, because of the extremely low amounts of Na^+ found in plants. Na^+ affects longevity and fecundity of some butterflies and survival of their eggs (Pivnick and McNeil, 1987), which may be true also in moths.

Only one cell responds to sucrose with positive monophasic action potentials (Fig. 3a) which become bipolar with slow return to baseline at higher concentrations or prolonged stimulation. Increasing concentrations of sucrose suppress the small NaCl spike. Sucrose cells in both types of sensilla exhibit essentially maximal response at 0.05 M sucrose (Fig. 3b, c). By comparison, stimulation of the tarsi with 0.02 M sucrose elicits proboscis extension in 50% of the individuals tested (Ramaswamy, 1987). The phasic sucrose response in both types of sensilla shows some adaptation within 100 msec (Fig. 3a), and is almost completely adapted after 1 sec of stimulation. It disadapts completely by 15 sec (Fig. 3a).

As a comparison, the 2 types of proboscis chemosensilla in TBW also exhibit a phasic response when exposed to 0.05 M sucrose (Blaney and Simmonds, 1988). There are no sexual differences in tarsal sensilla responses to sucrose, corroborating behavioral responses of TBW (Ramaswamy, 1987). Monophasic spikes similar to those in TBW were recorded from two other species of Lepidoptera. Tarsal receptors of *Pieris brassicae* responded to glucotropaeolin tetramethylammonium salt (GTA) with monopolar spikes (Ma and Schoonhoven, 1973); spike frequency increased with concentration of GTA but not with increasing NaCl concentration, a phenomenon

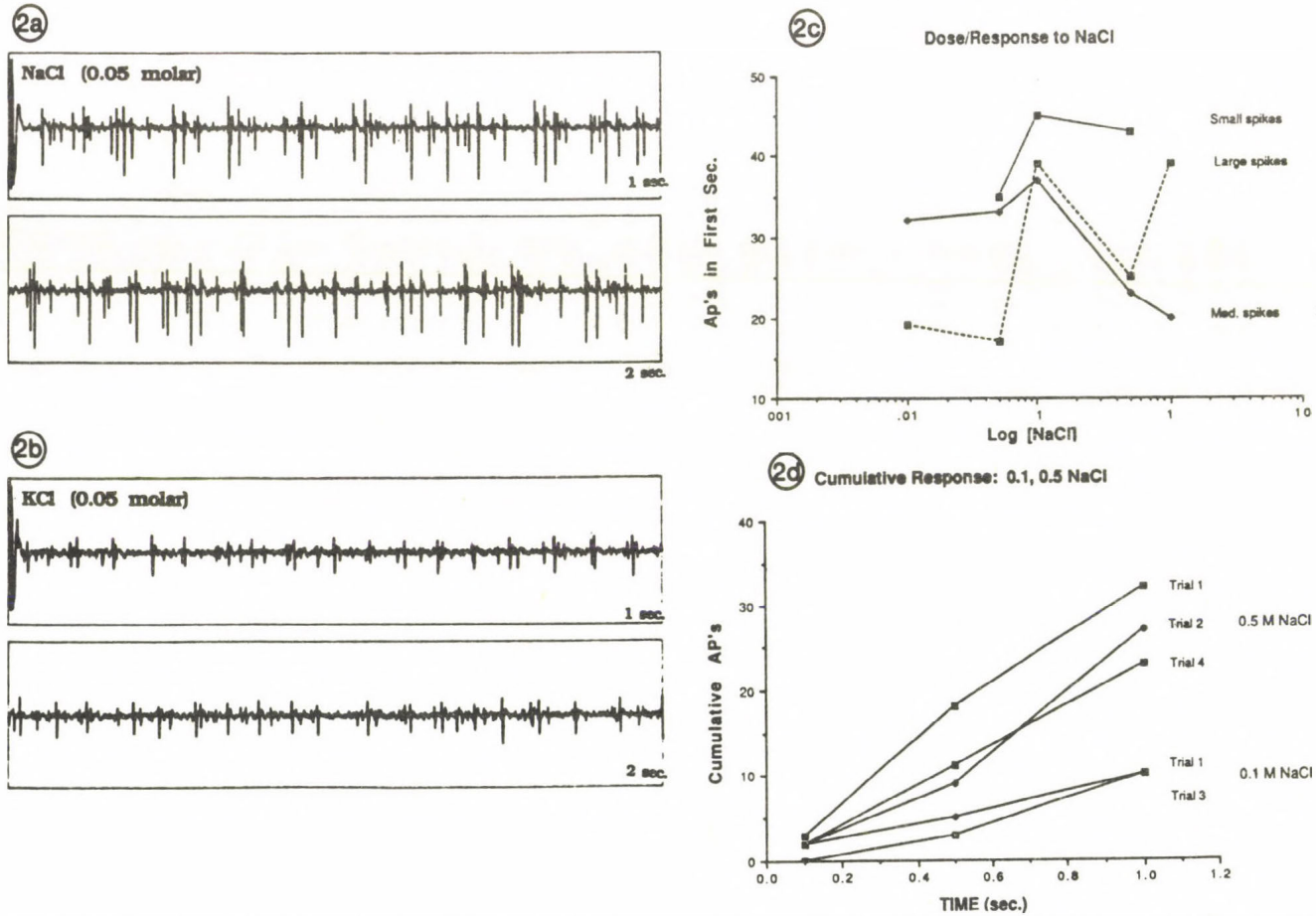

Fig. 2a Response of Type C sensillum to 0.05 M NaCl; *2b:* Response of same sensillum to 0.05 M KCl; *2c:* Dose/response of Type C sensillum to various concentrations of NaCl; *2d:* Cumulative response to 0.1 or 0.5 M NaCl over several trials to show adaptation to 0.5 M NaCl.

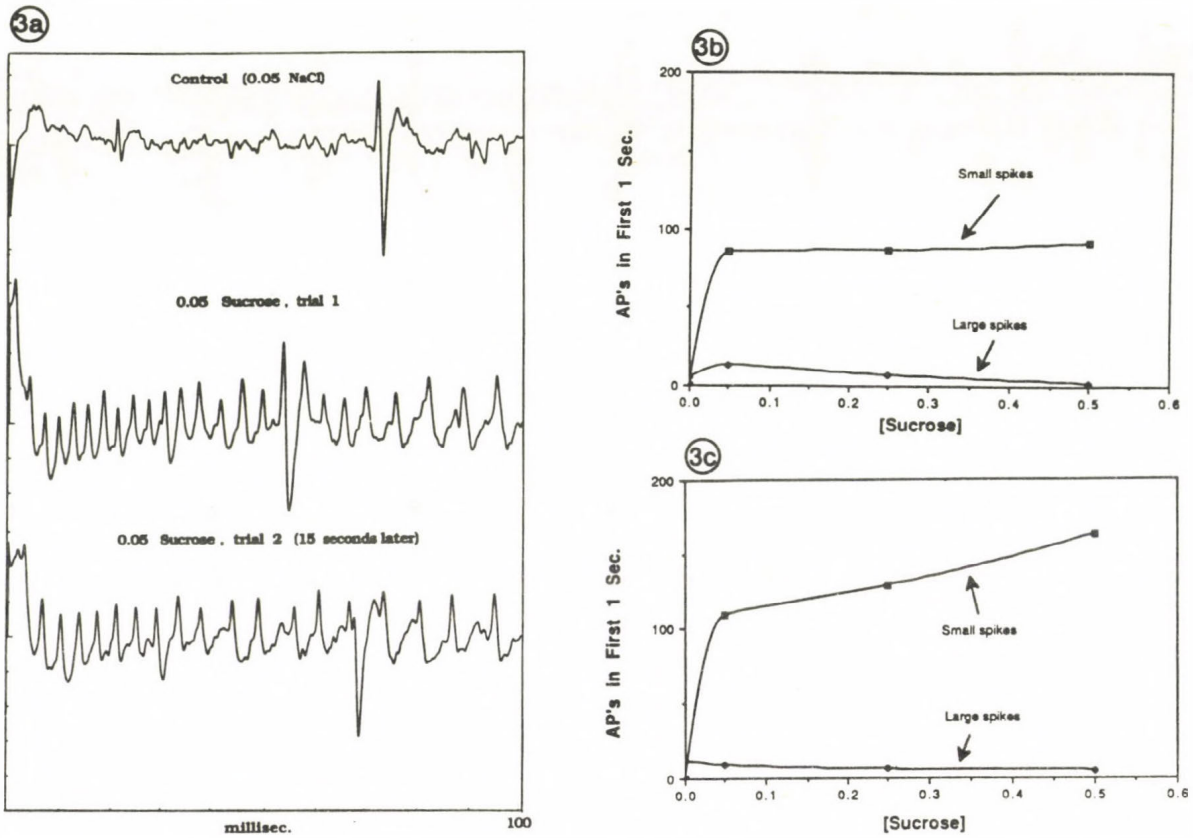

Fig. 3a. Response of Type C sensillum to 0.05 M NaCl followed 15 sec later by 0.05 M sucrose in 0.05 M NaCl; *3b* and *c:* Dose/responses of Type B and Type C sensilla to sucrose. Note that in *3b* and *3c,* "small spikes" refers to the monophasic spike of sucrose cells; "large spikes" refers to the NaCl cell.

similar to the sucrose and NaCl response in TBW. Lateral styloconic sensilla of *Manduca sexta* larvae also exhibit monophasic spikes when exposed to higher concentrations of quinine (J. L. Frazier, pers. comm.).

In preliminary studies, both types of sensilla responded to methanolic extracts of plants. Patterns of response to tobacco, cotton or ground cherry extracts are different (Fig. 4). Preliminary indications are that plant extracts stimulate salt cells. *H. virescens* uses tarsal chemo- and mechanosensory cues in host discrimination (Ramaswamy et al., 1987) and females may utilize ovipositor receptors to assess substrate suitability for oviposition (Ramaswamy, 1989). Future physiological studies on these sensilla should allow interpretation of their role in insect/host interactions.

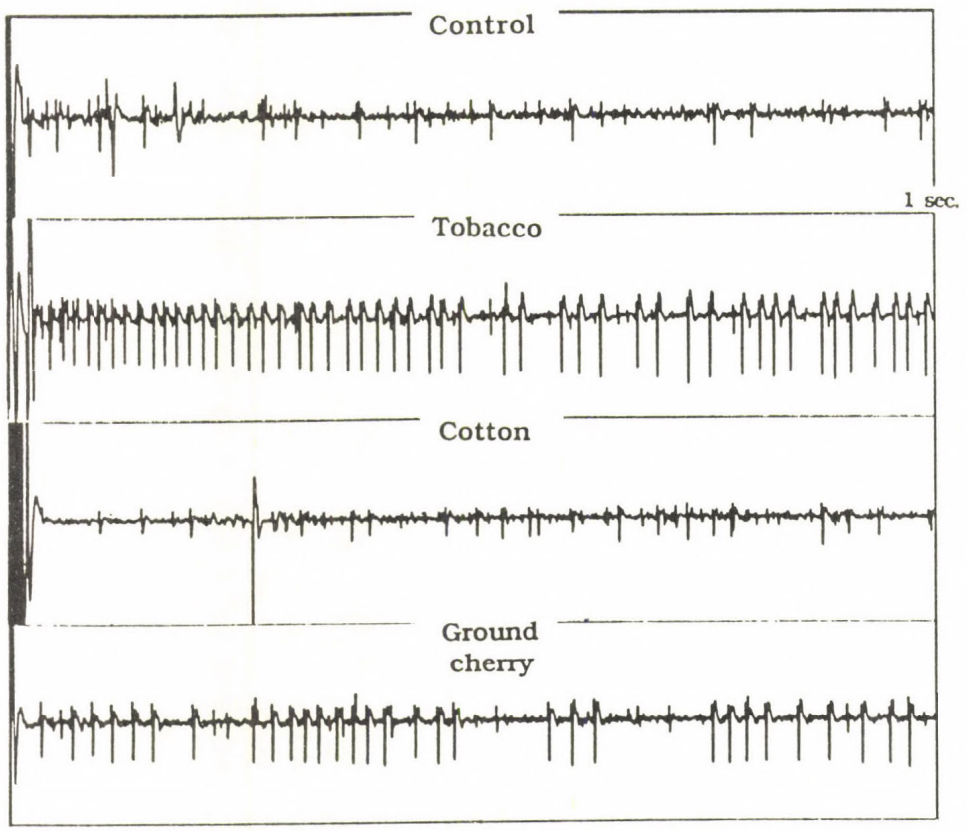

Fig. 4 Responses of Type B sensillum to 0.05 M NaCl ("Control") followed at 15 second intervals by stimulation with methanolic extracts of tobacco, cotton and ground cherry. Note that methanol was used also in the control and stimulus solutions.

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REFERENCES

Blaney, W. M. and M. S. J. Simmonds (1988): Food selection in adults and larvae of three species of Lepidoptera: a behavioural and electrophysiological study. *Entomol. exp. appl. 49,* 111-121.

Frazier, J. L. and F. E. Hanson (1986): Electrophysiological recording and analysis of insect chemosensory responses. In: J. R. Miller and T. A. Miller (eds), *Insect-Plant Interactions.* Springer-Verlag, New York. 285-330.

Hanson, F. E., S. Kogge and C. Cearley (1986): Computer analysis of chemosensory signals. In: T. L. Payne, M. C. Birch and C. E. J. Kennedy (eds), *Mechanisms in Insect Olfaction.* Oxford Univ. Press, Oxford. 269-278.

Ma, W-C. and L. M. Schoonhoven (1973): Tarsal contact chemosensory hairs of the large white butterfly *Pieris brassicae* and their possible role in oviposition behaviour. *Entomol. exp. appl. 16,* 343-357.

Navasero, R. C. and S. B. Ramaswamy (1989): Influence of plant surface characteristics on oviposition by *Heliothis virescens* (Lepidoptera: Noctuidae). *Ann. Appl. Biol.* (accepted).

Pivnick, K. A. and J. N. McNeil (1987): Puddling in butterflies: sodium affects reproductive success in *Thymelicus lineola. Physiol. Entomol. 12,* 461-472.

Ramaswamy, S. B. (1987): Behavioral responses of *Heliothis virescens* (Lepidoptera: Noctuidae) to stimulation with sugars. *J. Insect Physiol. 33,* 755-760.

Ramaswamy, S. B. (1988): Host finding by moths: sensory modalities and behaviours. *J. Insect Physiol. 34,* 235-249.

Ramaswamy, S. B. (1989): Periodicity of oviposition, feeding and calling by *Heliothis virescens* (Lepidoptera: Noctuidae). *J. Appl. Entomol.* (in press).

Ramaswamy, S. B., W. K. Ma and G. T. Baker (1987): Sensory cues and receptors for oviposition by *Heliothis virescens. Entomol. exp. appl. 43,* 159-168.

Waladde, S. M., H. M. Kahoro, E. D. Kokwaro and M. Chimtawi (1985): Responses of *Chilo partellus* to material obtained from susceptible and resistant maize cultivars. Electrophysiology and behaviour. *Insect Sci. Appl. 6,* 341-347.

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PLANT-SURFACE CHARACTERISTICS AND MOVEMENTS OF TWO BRASSICA-FEEDING APHIDS, *LIPAPHIS ERYSIMI* AND *BREVICORYNE BRASSICAE*

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ABSTRACT

Leaf waxiness hampered movements of the aphid *Lipaphis erysimi*. Fewer aphids reached their normal feeding sites on the underside of the leaves on waxy than on less waxy *Brassica* species. Leaf hairiness helped rather than hindered the aphids to move to the lower leaf surfaces.

L. erysimi is a serious pest on *Brassica campestris* and *B. juncea* grown as oilseeds in India. Plant breeding to increase waxiness of such oilseeds might reduce the aphid infestations, if performance of aphids restricted to upper leaf surfaces or fallen to the ground is poorer than performance of aphid at their normal feeding sites.

The movements of *Brevicoryne brassicae*, another pest of *Brassica*, was not affected by leaf surface characteristics like waxiness and hairiness.

Key words: waxiness, hairiness, plant resistance, *Lipaphis erysimi, Brevicoryne brassicae, Brassica.*

INTRODUCTION

Successful plant utilization by herbivorous insects may depend on surface characteristics of their host plants (Southwood, 1986). In *Brassica* crops there are several cases where glossy, "waxless", plant types have been found less infested by the two aphids *Brevicoryne brassicae* L. (the cabbage aphid) and *Lipaphis erysimi* Kalt. ssp. *pseudobrassicae* (the Indian mustard aphid) than the normal waxy ones (Thompson, 1963; Way and Murdie, 1965; Srinivasachar and Malik, 1972; Chatterjee and Sengupta, 1987). However, it is not clear what are the causes for these differences. Characters such as waxiness and hairiness can influence long- and close-range movements by the insect as well as the suitability of the plant in terms of microclimate, food and protection against natural enemies (Southwood, 1986).

In the present study, the movements of *L. erysimi* and *B. brassicae* were examined on a number of *Brassica* species differing in waxiness and hairiness. After landing on a plant aphids normally walk around and probe the plant surface. If the aphids stay on the plant they walk to sites where, presumably, the conditions are favourable for feeding and multiplication. Plant surface characteristics may, however, interfere with such movements.

MATERIAL AND METHODS

Test plants. Aphid behaviour was investigated on two different sets of *Brassica* spp. The species in the first set were selected to differ in leaf waxiness. The leaves of these plants were glabrous except for sparse hairs, mainly on leaf veins. The set consisted of *Brassica oleracea* L. fodder marrow-kale cv. Tema, *B. napus* L. summer oilseed rape cvs. Puma and Topas, *B. campestris* L. summer oilseed turnip rape cvs. Tyko and Sonja, yellow sarson cv. YSB-9, toria cv. T-9 and *B. juncea* (L.) Coss and Czern rai cv. Varuna. The plants in the second set all belong to the *B. oleracea*-group (Gustafsson, 1982) and were selected to range in hairiness from densely pubescent to glabrous. Two populations of *B. villosa, B. incana, B. rupestris,* one of *B. macrocarpa* and *B. oleracea* cv. Tema were used in the tests. *B. oleracea*-type of plants remained in the rosette stage whereas in course of time all the others started to flower and set pods under the prevailing greenhouse conditions (temp.: 20-23 °C; day length: 18 H by 400 W HQIE lamps; pot size: 8 x 10 cm ∅).

Aphid cultures. The aphids were reared in cages in the greenhouse, on the various *Brassica* spp. used in the tests. *B. brassicae* had been collected on *Brassica* oilseeds in Southern Sweden and *L. erysimi* on oilseeds in Haryana State, India, a few years prior to this study.

Testing procedures. One plant of each *Brassica* cultivar or population in the set was placed on a table in the green-house. Insect-trapping Stickem-Special® was glued around the petiole of one expanded leaf per plant. This hindered the aphids to leave the test leaf walking. A brown paper card (21 x 23 cm) with stickem glue around its edges was placed below the selected leaf, to collect aphids falling off. Five aphids were transferred to the upper side of each test leaf with a fine painting brush, 1-2 cm away from the leaf edge. The aphids' positions after 2 hours were recorded. About 5% of the aphids were not found on the leaf lamina or on the paper card and were excluded from the data analysis. Aphid behaviour was observed on both sets of plants in the rosette-flower stage. Moreover the set of glabrous plants was also tested at a time when all except cv. Tema were in the flower-pod stage. The aphids were then placed on the upper leaves of the plant. For each set of plants, and plant age of glabrous plants, there were 8 replicates with each of the two aphid species on 2-3 different plant specimens. In two of the replicates with young glabrous plants, apterous females were tested. Nymphs with wingbuds were used in all the other tests.

In addition, the inhibitory effect of leaf waxes on movements by *L. erysimi* was investigated in another series of trials. The upper side of a leaf of *B. oleracea* cv. Tema leaf was wiped with a soft paper towel to remove as much of the surface waxes as possible. Five aphids were transferred to such a leaf and another 5 to an adjacent, intact leaf on the same plant. Aphid movements were recorded as before in 12 replicates on 3 different plants with nymphs having wingbuds and in 8 replicates on 2 different plants with alate aphids.

Plant surface characterizations. To estimate the waxiness of glabrous plants, all the leaves equivalent to aphid-tested ones from 3 plants per variety were dipped in glass beakers with 150-300 ml of chloroform for 10 seconds. The solvent was evaporated

at room temperature and the residue weighed. To estimate leaf area, the dipped leaves were spread on paper sheets and photocopied. The weight of the cut-out paper leaves was transformed to leaf area.

Aphid-exposed leaves were cut off on the set of plants differing in pubescence and hair density was estimated under a microscope. Five to ten randomly selected 0.25 cm^2 or 1 cm^2 squares on the upper side of the leaves were examined; the smaller area and sample number being used if hair density was high. The length of 3 hairs per leaf was measured.

RESULTS

Waxiness. There were large differences in the amount of wax on the surface of the glabrous *Brassica* plants. In young plants, *B. oleracea* had the highest amounts, followed by *B. napus*, in its turn followed by *B. campestris* and *B. juncea* (Fig. 1a). At flower-pod stage, the upper leaves were more waxy than the lower ones (Fig. 1b). The species ranking in waxiness of lower leaves was the same as for young plants. As for the upper leaves, cv. Sonja was exceptionally waxy among the *B. campestris* cultivars and ranked even higher than *B. oleracea* and *B. napus* cultivars.

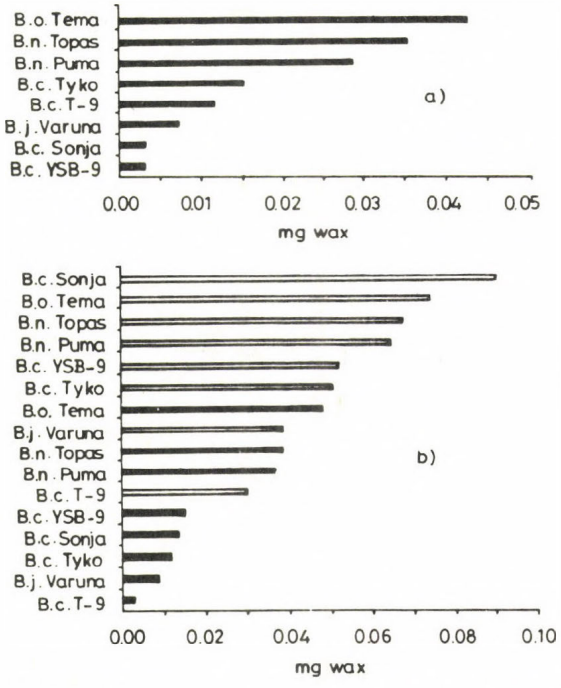

Fig. 1 Amount of wax per cm^2 leaf of *Brassica* cultivars. B.o. = *B. oleracea*, B.n. = *B. napus*, B.c. = *B. campestris*, B.j. = *B. juncea*. a) Leaves from plants in rosette-flower stage. b) Leaves from plants in flower-pod stage. White bars = upper leaves; black bars = lower leaves.

There was a significant, negative, correlation between the proportion of *L. erysimi* that had moved to the lower side of the leaves and amount of wax on the leaf surface of young plants (Fig. 2a). Even though *B. oleracea*, the plant with the most waxy leaves, also had the highest incidence of aphids falling off the leaf (25%), no linear relationship between waxiness and that type of aphid response was found. On older plants there was no linear relationship between the movements of *L. erysimi* on the upper leaves and leaf waxiness. In total, a higher proportion of aphids fell off in this test series, 25% compared to 7% in the previous test with young plants ($\chi^2 = 32.8$, $p < 0.001$). The highest proportion of *L. erysimi*, 47%, fell off the most waxy leaves, those of *B. campestris* cv. Sonja. Movements of *B. brassicae* were not linearly related to waxiness of the *Brassica* plants (Fig. 2a).

There was a significant difference in *L. erysimi* distributions on the wiped and the intact *B. oleracea* leaves. A higher proportion of aphids were found on the underside of those leaves where the wax had been removed (Table 1). The pattern was similar for alate females and nymphs with wingbuds.

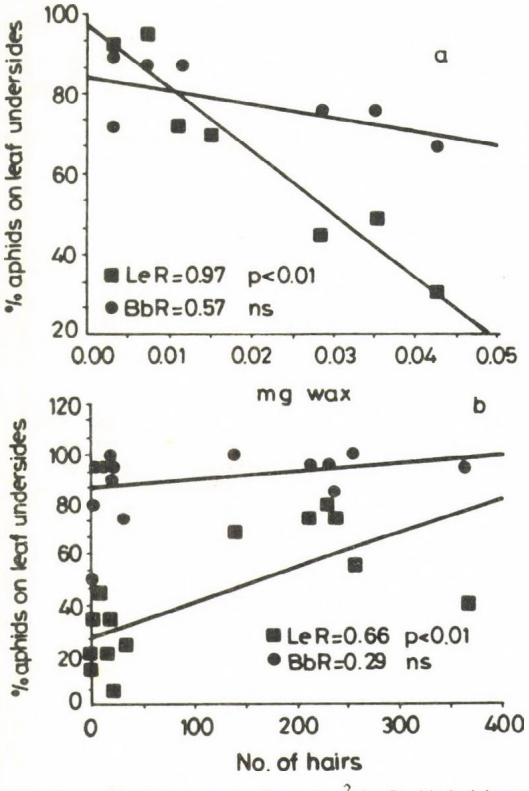

Fig. 2 Relationship between a) waxiness (amount of wax/cm^2 leaf), b) hairiness (hairs/cm^2 on leaf uppersides) and proportion of aphids found on the underside of the *Brassica* leaves 2 hours after the release. Le = *L. erysimi*, Bb = *B. brassicae*.

Table 1 Distribution of *L. erysimi* on intact *B. oleracea* leaves and leaves where the wax had been removed.

leaf type	alate females		nymphs with wingbuds	
	intact	wiped	intact	wiped
aphid position				
% on upperside	82.5	60.0	75.0	36.2
% on underside	7.5	37.5	16.7	60.3
% not on leaf lamina	10.0	2.5	8.3	3.4
n	40	40	60	58
2x3 contingency table	$x^2=11.2$ $p<0.01$		$x^2=23.9$ $p<0.001$	

Hairiness. B. macrocarpa and *B. oleracea* leaves had scattered hairs on veins and edges; *B. rupestris* had means of 10-31 hairs/cm^2; *B. villosa* had 211-255; and *B. incana* was variable carrying 6-364 hairs/cm^2 on leaf uppersides. The hairs were straight; 0.9-1.3 mm long on *B. rupestris, 0.5-0.6 mm* on *B. villosa* and 0.4-0.8 mm on *B. incana.*

Again *L. erysimi* was more sensitive than *B. brassicae* to the leaf surface characteristics. There was a significant, positive, linear relationship between proportion of *L. erysimi* that had moved to the underside of the leaves and hair density (Fig. 2b).

DISCUSSION

The movements of *L. erysimi* were hampered on waxy plant surfaces. Under natural conditions, weather and natural enemies may interact with effects of leaf characteristics on aphid movements to significantly affect the aphid performance. For example rainfall drastically reduces the populations of *L. erysimi* (e.g., Singh and Singh, 1982) and aphids on the upper sides of leaves are probably more easily washed off than aphids on the leaf undersides. Wind does probably also dislodge more aphids on slippery than on less slippery leaves. Aphids fallen to the ground may suffer from predation by ground-dwelling predators and they may also have difficulties to find new suitable host plants. Aphids staying on the upper leaf surfaces may experience an unfavourably hot or dry microclimate (Willmer, 1986) and natural enemies may detect aphids on leaf uppersides more easily. As the plants develop, *L. erysimi* changes its feeding position from leaves to inflorescences. Aphids among the flower buds of young plants are less easily dislodged by rain than aphids on branches and pods of older plants (Singh and Singh, 1982). Further, cultivars with buds and flowers densely packed, such as on *B. campestris,* are considered better for settlement by *L. erysimi* than others (Rai and Sehgal, 1975). Unfortunately the results from the present study on aphid movements do not give any further clues as to why certain glossy plant types have been found less infested by both *L. erysimi* and *B. brassicae* thant the normal, waxy ones.

Hairs on the *Brassica* leaves hindered neither of the two aphids *L. erysimi* and *B. brassicae* to reach the underside of the leaves. On the contrary, more *L. erysimi* were found on the leaf undersides of pubescent leaves.

In India, *L. erysimi* is a very severe pest on *B. campestris* and *B. juncea* grown as oilseeds (Bakhetia, 1987). Efforts are now being made in Sweden to develop waxier *B. juncea* cultivars by hybridizations with *B. oleracea. B. juncea* already has some other plant characters that cause *L. erysimi* populations to grow slower than on *B. campestris* (Bakhetia, 1987). Ultimately these research and breeding efforts need to be avaluated under the Indian field conditions.

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REFERENCES

Bakhetia, D. R. C. (1987): Insect pests of rapeseed-mustard and their management. In: M. V. Rao and S. Sithanantham (eds.) *Plant Protection in Field Crops* (Lead papers of the National Seminar on Plant Protection in Field Crops, 29-31 January, 1986, CPPTI, Hyderabad). 249-259.

Chatterjee, S. D. and K. Sengupta (1987): Observations on reaction of mustard aphid to white petal and glossy plants of Indian mustard. *J. Oilseeds Res. 4,* 125-127.

Gustafsson, M. (1982): Germplasm conservation of wild (n=9) Mediterranean *Brassica* species *Sveriges Utsädesfören. Tidskrift 92,* 133-142.

Rai, B. and V. K. Sehgal (1975): Field resistance of *Brassica* germplasm to mustard aphid *Liphaphis erysimi* (Kalt.) *Science and Culture 41,* 444-445.

Singh, R. and B. Singh. (1982): Influence of simulated rainfall on the population of mustard aphid (*Lipaphis erysimi*). *Indian J. Ecol. 9,* 344-345.

Southwood, T. R. E. (1986): Plant surfaces and insects — an overview In: B. Juniper and T. R. E. Southwood (eds), *Insects and the Plant Surface.* Edward Arnold (Publishers) Ltd., London. 1-22.

Srinivasachar, D. and R. S. Malik (1972): An induced aphid-resistant, non-waxy mutant in turnip, *Brassica rapa. Current Science 41,* 820-821.

Thompson, K. F. (1963): Resistance to the cabbage aphid (*Brevicoryne brassicae*) in *Brassica* plants. *Nature 198,* 209.

Way, M. J. and G. Murdie (1965): An example of varietal variations in resistance of Brussels sprouts. *Ann. Appl. Biol. 56,* 326-328.
Willmer, P. (1986): Microclimatic effects on insects at the plant surface In: B. Juniper and T. R. E. Southwood (eds), *Insects and the Plant Surface.* Edward Arnold (Publishers) Ltd., London. 65-80.

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DO PLANTS CRY FOR HELP? EVIDENCE RELATED TO A TRITROPHIC SYSTEM OF PREDATORY MITES, SPIDER MITES AND THEIR HOST PLANTS

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ABSTRACT

Plants may defend themselves against herbivores by attracting predators as bodyguards. Release of infochemicals to do so bears costs in terms of energy and eavesdropping risks. Therefore, selection pressure on release of a bodyguard attracting synomone is expected to have a minimization component. Recent evidence for herbivore induced release of a synomone provides an intriguing example of bodyguard recruitment, in which release of the information is minimized. Features of this phenomenon are described and costs for the plants are discussed.

Key words: Acarina, Phytoseiidae, *Phytoseiulus persimilis*, predators, tritrophic interactions, infochemicals, synomone, indirect defence, induced defence, energetic costs, terpenes, methylene terpene, phenol, evolutionary aspects, volatiles.

INTRODUCTION

One of the defence mechanisms of plants against herbivores is promoting effectiveness of the herbivores' natural enemies: natural selection will favour (1) plant genotypes coding for traits that promote effectiveness of natural enemies and (2) predator genotypes that are better able to use the opportunities offered by the plant (Price et al., 1980). Mutualistic interactions between plants and their herbivores' enemies are therefore to be expected.

To promote effectiveness of predators, plants may provide shelter or alternative food in times of prey scarcity (e.g., Pemberton and Turner, 1989; Hagen, 1986). This may retain predators that are already present. In addition, plants may also produce volatiles to attract their herbivores' natural enemies as bodyguards (e.g., Williams et al., 1988).

Apart from costs related to production of bodyguard attractants, this defence mechanism also bears costs in terms of eavesdropping. Unauthorized users, such as herbivore species that are not affected by the recruited bodyguards, may use the released cues as a means of locating a food source. Thus, both energetic costs and eavesdropping risks may constrain the duration of release of bodyguard attractants. Recent investigations provide evidence for production of a bodyguard attracting

infochemical (*sensu* Dicke and Sabelis, 1988) by plants which only starts after occurrence of herbivore damage (Dicke et al., 1989).

DISCUSSION

Plant volatiles and their information value to bodyguards. A plant that relies on predators as bodyguards for defence against herbivores will benefit from increasing the effectiveness of these protectors. This may be done by signalling of the plant's presence. If it is accomplished before herbivore damage occurs, the plant must provide the predator with some source of nutrition, e.g. in the form of pollen or extrafloral nectar (e.g. Hagen, 1986). Several examples of predator recruitment before herbivore damage occurs are known (e.g., Read et al., 1970, Williams et al., 1988). In all these examples the herbivores' natural enemies are parasitoids. In some instances a response towards odour of the plant's flowers, whose nectar is fed on by the parasitoids, has been observed (Shahjahan, 1974).

Attraction of predators may also occur through plant chemicals which end up in the products of the herbivore. For example, frass of the herbivore may well contain chemicals of plant origin which act as a kairomone. In some instances parasitoid response was found to depend on the herbivore's diet (Nordlund and Sauls, 1981; Elzen et al., 1984). This may be due to either non-modified plant compounds or to degraded plant compounds. Chemical analysis demonstrated that the former situation holds for the interaction between herbivorous larvae of the moth *Heliothis virescens* and the parasitic wasp *Campoletis sonorensis* (Elzen et al., 1984).

Plant volatiles and their information value to herbivores. Herbivores also use plant odours as infochemicals. This may occur in locating of suitable host plants, which is the more common response (for review see Visser, 1986) or in avoiding unsuitable host plants (Woodhead and Bernays, 1977; Gibson and Pickett, 1983). However, *non*-volatiles are well-documented for their role in avoiding unsuitable host plants (e.g., Schoonhoven, 1981). Thus, current evidence suggests that *volatile* plant infochemicals in plant-herbivore interactions are mostly to the detriment of the undamaged plant. It seems likely that herbivores in such cases spy on chemicals that the plants release for other purposes, such as recruitment of bodyguards.

Plant volatiles and their information value to nearby plants. Plants may use volatile pheromones from nearby conspecifics which have been damaged by herbivores (Baldwin and Schultz, 1983; Rhoades, 1983, 1985), but Fowler and Lawton (1985) criticized the results on methodological grounds. Recently, a convincing result has been reported by Zeringue (1987), who showed that cotton plants which receive odours of conspecifics infested with a pathogen start production of phenols which hamper pathogen growth. Neither odour of cultures of the pathogen on agar, nor odour of artificially damaged plants could elicit this reaction. The plants rely on information about increased risks of pathogen infection, instead of producing phenolic compounds constitutively. The success of this strategy depends on detecting increased chances of infestation at an early stage. Unless neighbouring plants in

nature show a high degree of relatedness it is unlikely that the infested plant would produce the pheromone with the aim of informing conspecifics.

Costs of volatiles to plants. Chemicals used in defence have costs in terms of production, transport, storage, prevention of autotoxication, and release. In addition, there are also maintenance costs to synthesize enzymes needed in the above-mentioned processes. Calculation of overall costs is difficult, for several of the component costs. Biosynthetic costs are the component that can be usually quantified most reliably (Chew and Rodman, 1979). Intuitively, volatiles seem to constitute an extra cost for plants, since they are lost upon release and thus have to be renewed more often than non-volatiles. Several characteristics of volatile-release support this view:

(1) Plant volatiles are usually released in minute quantities and can only be analysed with very sensitive collection and analysis techniques (e.g., Buttery et al., 1984). The quantities released are in the ppm range, although upon damage, volatiles are generally released in much higher quantities (e.g., Dicke, 1988).

(2) Several volatiles are only released upon damage. For instance, precursors may be stored and degraded enzymatically when compartmentation is destroyed by herbivory, as is the case for production of volatiles from cyanogenic glycosides (Woodhead and Bernays, 1977; Conn, 1979).

(3) Release may be dependent on time of the day. For instance, some flower fragrances are produced exclusively during the day or the night, which is correlated with activity of their pollinators (Altenburger and Matile, 1988; Harborne, 1988). It would be a waste of energy for the plant to release volatiles while the pollinators are inactive and it would only increase chances of being located by herbivores.

These characteristics indicate that it is worthwile for a plant to minimize its volatile release because of energetic costs and risks of use by "unauthorized receivers". The induced release of volatiles is a major means of reducing both types of costs. This is known for plant-herbivore interactions (Woodhead and Bernays, 1977; Harrison and Karban, 1986), but recently evidence was obtained that induced release of volatile infochemicals also occurs in plant-predator interactions (see below).

Induced indirect defence in a system consisting of predatory mites, herbivorous mites and their host plants. Spider mites are polyphagous herbivores that reach pest status in many agricultural crops (see Helle and Sabelis, 1985a for review). They insert their stylets in the leaves, inject saliva and ingest parenchymous cell contents. Spider mites are particularly ravenous herbivores, overexploiting their food source in the absence of predators. However, local populations are decimated, if discovered by predators such as predatory mites (see Helle and Sabelis, 1985b for review). Long-range dispersal by predatory mites occurs on wind currents. The aeronauts probably cannot control where they land and thus, chances of landing in a spider-mite colony or on a spider-mite infested plant will be small. However, after landing volatile infochemicals are used in making foraging decisions such as whether to stay or take off again, and where or how long to search (for review see Sabelis and Dicke, 1985). Because predatory mites overexploit their prey locally, it may be envisaged that any plant genotype that increases chances of predatory-mite invasion into spider-mite colonies will have a relatively higher fitness than conspecifics without this ability.

Much research has been done on a tritrophic system consisting of the predatory mite *Phytoseiulus persimilis*, the spider mite *Tetranychus urticae* and its host plants. Predatory mites distinguish between clean plants and spider-mite infested plants by means of olfaction. The volatile allelochemical involved is mainly emitted from the leaves after infestation. Upon removal of spider mites and their visible products, previously infested leaves remained attractive to predatory mites for several hours, whereas the spider mites removed from the leaves were not attractive (Sabelis and Van de Baan, 1983; Sabelis et al., 1984a). The attractiveness is correlated with the amount of feeding by spider mites (Sabelis and Van de Baan, 1983; Sabelis et al., 1984a). Behavioural investigations in which the components of spider-mite infested plants were separately tested in an olfactometer showed that the volatile allelochemical is a product of the interaction between spider mites and host plant (Sabelis et al., 1984a). Chemical analysis revealed only compounds that are well-known from the plant kingdom, that are not known to be produced *de novo* by animals (Dicke et al., 1989). The allelochemical is spider-mite species specific (Sabelis and Van de Baan, 1983) but it is interesting to see that it is also plant-species specific (Takabayashi and Dicke, in prep). For example, cucumber plants infested by two-spotted spider mites are much less attractive than Lima bean plants infested by this herbivore species (Takabayashi et al., in prep). Different mixtures of volatiles are emitted by these plant species when infested by two-spotted spider mites. Four of the compounds emitted by infested Lima bean plants are attractive: the terpenes linalool and (*E*)-β-ocimene, the methylene terpene 4,8-dimethyl-1,3(*E*),7-nonatriene and the phenolic compound methyl salicylate (Dicke et al., 1989), whereas only two of these (ß-ocimene and the methylene terpene) are emitted by infested cucumber plants (Takabayashi et al., in prep).

Apart from this evidence for plant involvement in production of the allelochemical (a synomone if plant-predatory mite interactions are regarded or a kairomone when spider mite-predatory mite interactions are regarded; cf. Dicke and Sabelis, 1988), behavioural investigations indicate that its production is not restricted to infested leaves but occurs plant-wide: uninfested leaves of infested plants are more attractive than uninfested leaves of uninfested plants. In these experiments adsorption of synomone to uninfested leaves of infested plants has been precluded (Dicke et al., in prep).

Effect of induced infochemical on spider-mite behaviour. Tetranychus urticae is repelled by leaves with a high density of conspecifics. The involved volatile infochemical overlaps with the synomone which attracts predatory mites, in at least one component: linalool (Dicke, 1986; Dicke et al., 1989). This interaction between spider mites raises the question whether (1) predatory mites spy on spider-mite communication or (2) spider mites leave as soon as the plant's cry for help gets too loud. This is essentially a question about who controls production and/or release of the chemicals.

1. The spider mites might control production of the infochemical to inform conspecifics about local density, and thus about food quantity and prospects for competition. But the spider mites would not need a volatile compound for this because information can also be conveyed by non-volatile chemicals or by contacts between

individuals, without the costs of volatiles or the associated risks of attracting predatory mites.

2. The plant might control production of volatiles to recruit bodyguards. The volatile nature of the chemicals is then indispensable for fast spread of the advertisement: success of *induced* indirect defence depends heavily on rapidly recruited defenders. In this case the spider mites would do best by using the volatiles as a dispersing pheromone. It seems unlikely that the mites could avoid the feeding-dependent release of the infochemical, unless they can make their feeding unrecognizable for the plant.

These considerations suggest that the volatile infochemical primarily functions in plant-predatory mite interactions and that the spider-mite response is secondary.

Bodyguard attracting synomone and spider mite extermination. Simulation models of the local population dynamics of the system consisting of predatory mites, spider mites and their host plants gave exceedingly bad predictions when the predators were assumed to search at random as they do in prey-infested leaf areas. However if the predators, upon leaving the prey-infested leaf area, are assumed to return immediately, the simulation models gave reasonably good predictions (Sabelis and Van der Meer, 1986). Behavioural studies have demonstrated that the volatile-synomone gradient present at the edge of the patch affects such returning behaviour (Sabelis et al., 1984b) and that, even when starved, the predatory mite *P. persimilis* did not take off on wind currents as long as the volatile infochemical was present (Sabelis and Afman, 1984). Because the volatile synomone appears to be essential in spider-mite extermination it is important to study the role of the plant in its production in more detail, thus improving knowledge of this indirect inducible defence mechanism.

Costs of bodyguard recruitment. In a first attempt to estimate biosynthetic costs of the synomone, Dicke and Sabelis (1989) reported a conservative estimate of 0.001% of leaf production costs per day. This value may seem to be low, but biosynthetic costs are but one of the costs made. Other costs may comprise e.g. maintenance costs for synthesizing enzymes, or costs of transport and storage of synomone (precursors). Moreover, even if overall costs are low indeed, their impact should not be overlooked since, given conditions of exponential leaf growth, small costs in an early phase may have large consequences for the production of reproductive tissue in the end (Gulmon and Mooney, 1986). It is also important to note that production of *volatile* infochemicals is much more expensive than production of non-volatiles, because volatiles have to be renewed constantly.

The view that costs are not negligible is strenghtened by the fact that plants do not produce the infochemical non-stop. Eavesdropping risks may be responsible as well, but no evidence is available yet for this system.

When costs related to synomone production may be offset by saving the energy for other fitness-related purposes and by the probability of settlement close to a synomone producing plant, polymorphism is expected to occur under a wide range of conditions (Sabelis and De Jong, 1988).

Future investigations will concentrate on *how* plants contribute to synomone production, on cost-reducing mechanisms that plants may employ and on the use of the released information by each of the three trophic levels.

REFERENCES

Altenburger, R. and P. Matile (1988): Circadian rhythmicity of fragrance emission in flowers of *Hoya carnosa* R. Br. *Planta 174,* 248-252.

Baldwin, I. T. and J. C. Schultz (1983): Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants. *Science 221,* 277-279.

Buttery, R. G., J. A. Kamm, and L. C. Ling (1984): Volatile components of red clover leaves, flowers, and seed pods: possible insect attractants. *J. Agric. Food Chem. 32,* 254-256.

Chew, F. S. and J. E. Rodman (1979): Plant resources for chemical defense. In: G. A. Rosenthal and D. H. Janzen (eds), *Herbivores. Their Interaction with Secondary Plant Metabolites,* Academic Press, New York. 271-307.

Conn, E. E. (1979): Cyanide and cyanogenic glycosides. In: G. A. Rosenthal and D. H. Janzen (eds,: *Herbivores. Their Interaction with Secondary Plant Metabolites,* Academic Press, New York. 387-412.

Dicke, M. (1986): Volatile spider-mite pheromone and host-plant kairomone, involved in spaced-out gregariousness in the spider mite *Tetranychus urticae. Physiol. Entomol. 11,* 251-262.

Dicke, M. (1988): Infochemicals in Tritrophic Interactions. Origin and Function in a System Consisting of Predatory Mites, Phytophagous Mites and Their Host Plants. *Ph.D. Thesis, Agricultural University Wageningen,* The Netherlands.

Dicke, M. and M. W. Sabelis (1988): Infochemical terminology: Should it be based on cost-benefit analysis rather than origin of compounds? *Funct. Ecol. 2,* 131-139.

Dicke, M. and M. W. Sabelis (1989): Does it pay plants to advertize for bodyguards? Towards a cost-benefit analysis of induced synomone production. In: Lambers, H., H. Konings, M. L. Cambridge and Th. L. Pons (eds), *Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants.* SPB Academic Publishing bv., The Hague.

Dicke, M., T. van Beek, M. A. van Posthumus, N. Ben Dom, H. van Bokhoven, and Æ. de Groot (1989): Isolation and identification of volatile kairomone that affects acarine predator prey interactions: involvement of host plant in its production. *J. Chem. Ecol. 15,* (in press).

Elzen, G. W., H. J. Williams and S. B. Vinson (1984): Role of diet in host selection of *Heliothis virescens* by parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *J. Chem. Ecol. 10,* 1535-1541.

Fowler, S. V. and J. H. Lawton (1985): Rapidly induced defences and talking trees: the devil's advocate position. *Am. Nat. 126,* 181-195.

Gibson, R. W. and J. A. Pickett (1983): Wild potato repels aphids by release of aphid alarm pheromone. *Nature 302,* 608-609.

Gulmon, S. L. and H. A. Mooney (1986): Costs of defense and their effects on plant productivity. In: T. J. Givnish (ed), *On the Economy of Plant Form and Function,* Cambridge University Press, Cambridge. 681-698.

Hagen, K. S. (1986): Ecosystem analysis: Plant cultivars (HPR), entomophagous species and food supplements. In: D. J. Boethel and R. D. Eikenbary (eds), *Interactions of Plant Resistance and Parasitoids and Predators of Insects,* Ellis Horwood, Chichester. 151-197.

Harborne, J. B. (1988): *Introduction to Ecological Biochemistry.* 3rd Edition, Academic Press, London.

Harrison, S. and R. Karban (1986): Behavioural response of spider mites (*Tetranychus urticae*) to induced resistance of cotton plants. *Ecol. Entomol. 11,* 181-188.

Helle, W. and M. W. Sabelis (1985a): *Spider Mites. Their Biology, Natural Enemies and Control.* World Crop Pests, Vol. 1A. Elsevier, Amsterdam.

Helle, W. and M. W. Sabelis (1985b): *Spider Mites. Their Biology, Natural Enemies and Control.* World Crop Pests Vol. 1B. Elsevier, Amsterdam.

Nordlund, D. A. and C. E. Sauls (1981): Kairomones and their use for management of entomophagous insects. XI. Effect of host plants on kairomonal activity of frass from *Heliothis zea* larvae for the parasitoid *Microplitis croceipes. J. Chem. Ecol. 7,* 1057-1061.

Pemberton, R. W. and C. E. Turner (1989): Occurrence of predatory and fungivorous mites in leaf domatia. *Amer. J. Bot. 76,* 105-112.

Price, P. W., C. E. Bouton, P. Gross, B. A. McPheron, J. N. Thompson and A. E. Weis (1980): Interactions among three trophic levels: influence of plant interactions between insect herbivores and natural enemies. *Ann. Rev. Ecol. Syst. 11,* 41-65.

Read, D. P., P. P. Feeny and R. B. Root (1970): Habitat selection by the aphid parasite *Diaeretiella rapae* (Hymenoptera: Braconidae) and hyperparasite *Charips brassicae* (Hymenoptera: Cynipidae). *Can. Entomol. 102,* 1567-1578.

Rhoades, D. F. (1983): Responses of alder and willow to attack by tent caterpillars and webworms: Evidence for pheromonal sensitivity of willows. In: P. A. Hedin (ed), *Plant Resistance to Insects,* Washington DC. 55-68.

Rhoades, D. F. (1985): Pheromonal communication between plants. *Recent Advances in Phytochemistry, 19,* 195-218.

Sabelis, M. W. and B. P. Afman (1984): Factors initiating or suppressing aerial dispersal of the predatory mite *Phytoseiulus persimilis. Abstr. 17th Int. Cong. Entomol.,* Hamburg, August 1984. 445.

Sabelis, M. W. and H. E. van de Baan (1983): Location of distant spider mite colonies by phytoseiid predators: demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi. Entomol. exp. appl. 33,* 303-314.

Sabelis, M. W. and M. Dicke (1985): Long-range dispersal and searching behaviour. In: W. Helle and M. W. Sabelis (eds), *Spider Mites. Their Biology, Natural Enemies and Control.* World Crop Pests, Vol. 1B, Elsevier, Amsterdam. 141-160.

Sabelis, M. W. and M. C. M. de Jong (1988): Should all plants recruit bodyguards? Conditions for a polymorphic ESS of synomone production in plants. *Oikos 53,* 247-252.

Sabelis, M. W. and J. van der Meer (1986): Local dynamics of the interaction between predatory mites and two-spotted spider mites. In: J. A. J. Metz and O. Diekmann (eds), *Dynamics of Physiologically Structured Populations,* Lecture Notes in Biomathematics, Springer, Berlin. 322-343.

Sabelis, M. W., B. P. Afman and P. J. Slim (1984a): Location of distant spider mite colonies by *Phytoseiulus persimilis*: localization and extraction of a kairomone. *Acarology VI,* Vol 1, 431-440.

Sabelis, M. W., J. E. Vermaat and A. Groeneveld (1984b): Arrestment responses of the predatory mite, *Phytoseiulus persimilis*, to steep odour gradients of a kairomone. *Physiol. Entomol. 9,* 437-446.

Schoonhoven, L. M. (1981): Chemical mediators between plants and phytophagous insects. In: D. A. Nordlund, R. L. Jones and W. J. Lewis (eds), *Semiochemicals. Their Role in Pest Control.* Wiley and Sons, New York. 31-50.

Shahjahan, M. (1974): Erigeron flowers as a food and attractive odor source for *Peristenus pseudopallipes*, a braconid parasitoid of the tarnished plant bug. *Environ. Entomol. 3,* 69-72.

Visser, J. H. (1986): Host odor perception by phytophagous insects. *Ann. Rev. Entomol. 31,* 121-144.

Williams, H. J., G. W. Elzen and S. B. Vinson (1988): Parasitoid-host-plant interactions, emphasizing cotton (*Gossypium*). In: P. Barbosa and D. K. Letourneau (eds), *Novel Aspects of Insect-Plant Interaction. Wiley and Sons, New York. 171-200.*

Woodhead, S. and E. A. Bernays (1977): Changes in release rates of cyanide in relation to palatability of *Sorghum* to insects. *Nature 270,* 235-236.

Zeringue, H. J. Jr. (1987): Changes in cotton leaf chemistry induced by volatile elicitors. *Phytochem. 26,* 1357-1360.

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ROLE OF BEHAVIOUR OF THE STEM-BORER *CHILO PARTELLUS* (SWINHOE) IN DETERMINING RESISTANCE OR SUSCEPTIBILITY OF CERTAIN SORGHUM CULTIVARS

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ABSTRACT

The behaviour of an insect plays an important role in selecting or rejecting a plant for colonization. 3 types of behaviour are most important in this respect: orientation, feeding and oviposition . The role of these responses of the sorghum stem borer *Chilo partellus* in determining the relative resistance or susceptibility of three different cultivars to the pest has been investigated: susceptible IS 18363, tolerant IS 18520, resistant IS 1044. The sequence in which different behavioural responses of the stem borer determine the differences in its colonization of the above cultivars is as follows: (i) *orientation* of adults (ii) *oviposition*, after arrival, on a plant; (iii) *orientation* of the emerging 1st instar larvae involving their (a) arrest and settling on the plant within the whorl or dispersal therefrom, and (b) *attraction* of the dispersing larvae resulting in their arrival on another plant; (iv) feeding and development within leaf whorl during 3 instars; (v) movement of late 3rd or early 4th instar larvae out of the whorl, followed by their arrest and boring into the stem of the same plant or dispersal and attraction to another plant; (vi) feeding and development during 4th-6th instars within the stem, leading to final emergence of adults to repeat the above sequence. Differences have been observed between the test cultivars in respect of above behavioural responses of *C. partellus* and their relative contributions to the susceptibility or resistance of the cultivars have been elucidate.

Key words: Chilo partellus, sorghum, plant resistance, orientation, oviposition, attraction.

INTRODUCTION

The stem borer *Chilo partellus* (Swinhoe) is a major pest of sorghum in Africa and southern Asia. Different cultivars of the crop differ in their susceptibility or resistance according to their suitability for colonization. Behaviour is among those factors which determine pest colonization and hence the susceptibility/resistance of a cultivar to a pest. Saxena (1985) distinguished 3 broad categories of behaviour:

(i) Orientation which determines an insect's arrival and stay on a plant due to attraction and arrest, or its avoidance due to repulsion or lack of attraction,
(ii) feeding; and
(iii) oviposition.

Since it is an interaction of these responses, rather than any one of them in isolation, that determines the resistance or susceptibility of a cultivar, it is important to compare all these responses to the same cultivars. Our knowledge in this respect

is quite limited. For example, the oviposition response of *C. partellus* to some sorghum cultivars has been studied without comparing any other behavioural response (Lal and Pant, 1980; Dabrowski and Kidiavai, 1983; Singh and Rana, 1984). On the other hand, some information is available on the orientation of early larval instars of *C. partellus* in reports on their pattern of movement on other cultivars (Roome, 1980; Bernays et al., 1983; Chapman et al., 1983). But, these movements have been studied under conditions for which no information on oviposition or feeding is available. Feeding responses based on direct measurement of consumption have hardly been compared among these cultivars. In order to cover these gaps in our knowledge, the above mentioned behavioural responses of *C. partellus* to a susceptible, a tolerant and a resistant sorghum cultivar have been compared and the results are presented in this paper.

MATERIALS AND METHODS

These studies were carried out at the ICIPE's Field Station at Mbita on the shores of Lake Victoria in western Kenya. A culture of *C. partellus* was maintained on an artificial diet (Ochieng et al., 1985). Three sorghum cultivars were used: IS 18363 (susceptible), IS 18520 (tolerant) and IS 1044 (resistant). The plants were grown in the field as described later. The temperature during the field tests and the laboratory tests was 24°-30°C with r.h. 60-80%, and 23°-26°C with r.h. 60-70%, respectively.

Oviposition responses. Methods were designed to avoid variation in the number of eggs laid on different cultivars due to non-plant factors, e.g. female population, mating status, fecundity, other physiological conditions and environmental factors like temperature, humidity, light intensity, etc.

The tests were conducted in a 3-sector chamber (210 x 80 x 80 cm) with a glass-walled central sector between, and continuous with, two terminal sectors walled with wire mesh as described previously (Saxena, 1987). The roof was of glass and the bottom was formed by the floor of the test arena. Three plants of one cultivar were grown in a row within, and along the end-wall of, one terminal sector. Waxed paper sheets were stuck on the end wall of the opposite terminal sector and served as the 'blank', non-plant ovipositional substrate. Five 1-day-old, mated females were released in the central sector at dusk. The number of eggs laid overnight on the plants and on the 'blank' were recorded next morning. The tests were repeated 5 times with different batches of insects.

Larval arrest and dispersal. The test plants were each infested 3-5 weeks after emergence (WAE) with 20 neonate 1st instar larvae and 5-7 WAE with single, freshly moulted 4th instar larvae in the field on outermost leaves of the whorls. Tests with 1st instar larvae were repeated 5 times, and those with 4th instar larvae on 30 plants arranged in 3 replicates of 10 plants each. The larvae were observed for 1 hr and then after 72 h (1st instar) or 24 h (4th instar), the plants were dissected. The percentage of the introduced larvae that were still present were recorded and reflected larval arrest.

In another test series, 10 neonate 1st instar larvae were released 10 each of 10 plants of: (i) the susceptible cultivar (IS 18363) in a row between two rows of an equal number of plants of the resistant cultivar (IS 1044), or (ii) a row of the resistant cultivar between two rows of the susceptible cultivar. The rows were 60 cm apart, with 30 cm between plants. The plants were dissected after 24 h and the percentages of larvae that remained were recorded. The tests were replicated 5 times.

Larval attraction. Sheets of filter paper (60 cm dia.) were spread flat around single sorghum plants in the field. Forty neonate 1st instar, or 5 freshly moulted 4th instar, larvae were released around a plant, 30 cm from its base. The percentages of larvae that reached the plant and those that moved off the filter paper sheet in 30 min were recorded; the former reflected the plant's attractancy. Tests with 1st instar larvae were replicated three times and those with 4th instar larvae 5 times.

In another series of tests with 1st instar larvae, the plants were grown in a 3 x 3 m plot consisting of 5 rows, spaced as above, planted parallel to the direction of the prevailing wind. A rectangular tray (35 x 25 cm) with vertical edges of 10 cm on the longer sides was placed 20 cm from the downwind end of the plot in line with the central row of plants. Twenty neonate larvae were released across the tray, mid-way along it. The percentages that moved towards the plants and away from them were recorded. These tests were replicated 5 times with different batches of larvae. A greater percentage of larvae moving towards plants, rather than towards the opposite end, reflected the attractancy of the plants.

Larval feeding. Twenty 1st instar larvae were offered a 7-cm basal segment of a leaf whorl, and single 4th instar larvae a 7-cm basal, middle or top internode segment. The leaf area consumed in 72 h and volume of stem segments consumed in 24 h were measured and compared among the cultivars. Each test was replicated 5 times.

RESULTS AND DISCUSSION

The sequence of various major colonizing responses of *C. partellus* to sorghum is diagrammatically shown in Fig. 1. Steps 1-7 and 10-13 represent behavioural responses which we have compared among the test cultivars.

Oviposition responses. As shown in Fig. 1, the initial selection (steps 1 and 2) of a plant by *C. partellus*, like many other lepidopterans, is done mostly by adult females for oviposition. Differences in these responses to different cultivars can contribute to their susceptibility or resistance. The number of eggs laid by the females on the plants of each cultivar, offered alone, was significantly higher than that on the blank substrate (Fig. 2). This indicates that all the cultivars were more suitable for oviposition than waxed paper, though the latter has been reported to be a suitable in the absence of plants (Kumar and Saxena, 1985). However, the number of eggs was laid on plants of the susceptible cultivar, and lowest for the resistant cultivar. Of the egss laid on the plants, the percentage on the resistant cultivar was significantly lower than for the other two. This suggests that a lower oviposition response of *C. partellus* to IS 1044 can contributes to the higher resistance of this cultivar.

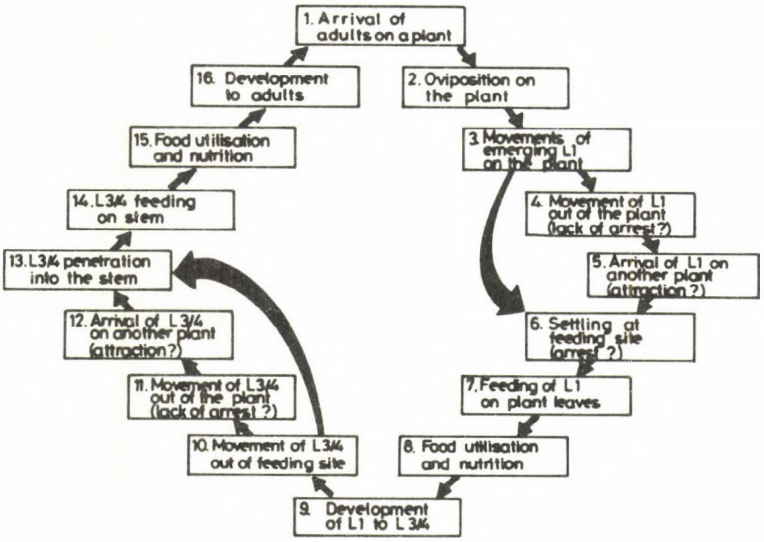

Fig. 1 Sequence of responses of *Chilo partellus* leading to its colonization of sorghum plants. L1: 1st instar larva; L3/4: late 3rd or early 4th instar larva.

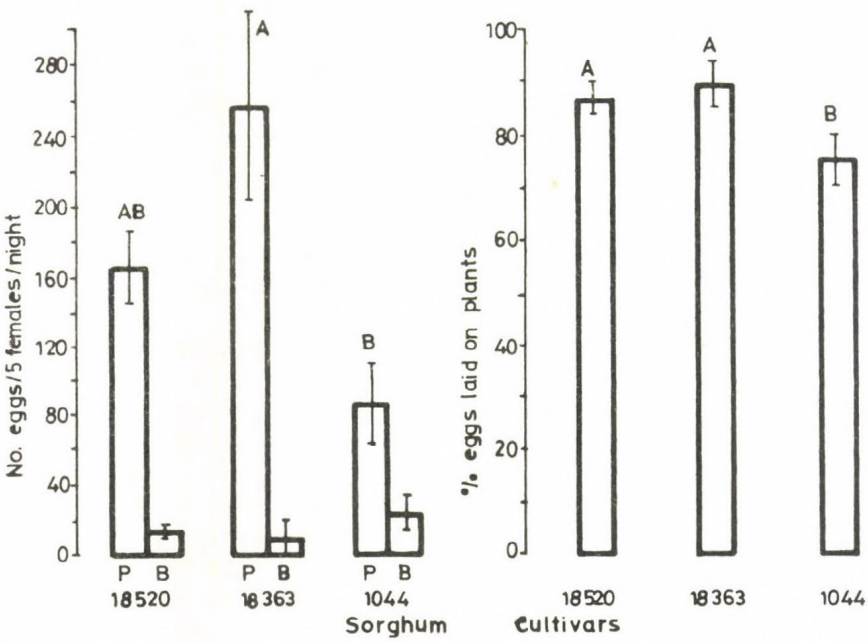

Fig. 2 Ovipositional responses of *Chilo partellus* to three sorghum cultivars (3-5 weeks old), each (P) presented as a choice against waxed paper blank (B). The columns bearing different letters on top differ significantly at P=0.05 (ANOVA/DMRT after arcsine transformation).

Table 1 Arrest and dispersal of 1st instar larvae of *Chilo partellus* released on a susceptible and a resistant sorghum cultivar grown in the field in a row between two rows of the other cultivar[1]

Cultivar[2]	% Larvae recovered after 24 h (Mean±s.e. of 5 replicates each)	
	IS 18363	IS 1044
IS 18363 (susceptible)	46.8±2.0 Aa[3]	16.3±1.5 Ab
IS 1044 (resistant)	25.3±5.8 Bb	19.8±3.5 Aa

[1] All plants 3-5 weeks old.
[2] 100 larvae released, 10 on each of 10 plants in the middle row.
[3] Means in each column followed by a different capital letter, and means in each row followed by a different small letter, are significantly different at P=0.05 (ANOVA/DMRT after arcsine transformation).

Larval arrest, dispersal and attraction in the first instar. When the eggs hatch, the emerging larvae move about in various patterns (Fig. 1, step 3) which may lead them off the plant to disperse, or into the leaf whorl to settle and feed (steps 6, 7). The proportion of larvae settling on a plant depends on its suitability for larval arrest, which was high for IS 18363 and IS 18520 (Fig. 3) but was about one-third less on IS 1044, and this could contribute to its resistance.

The larvae that disperse may arrive on other plants either by chance or as a result of definite attraction (Fig. 1, steps 4, 5). The percentage of 1st instar larvae that moved towards a plant in a 5-row plot was higher than that to single plants of the same cultivar (Fig. 3). This was evidently due to a greater attractiveness of the grouped plants. Nevertheless, the percentage of larvae attracted to IS 1044 was much lower than towards IS 18363 in both situations, and would contribute to the resistance of the former cultivar.

Of the 1st instar larvae released on IS 18363 plants grown row between two rows of IS 1044, the percentage remaining on IS 18363 after 24 h was significantly higher

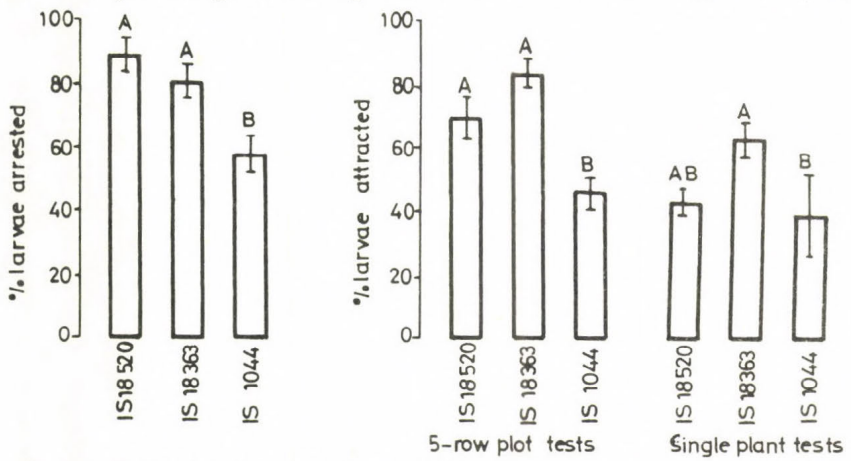

Fig. 3 Arrest and attraction of 1st instar larvae of *C. partellus* by three sorghum cultivars (3-5 weeks old). The columns bearing different letters on top for a given series of tests differ significantly from one another at P=0.05 (ANOVA/DMRT after arcsine transformation).

Table 2 Site of entry of 4th instar larvae of *Chilo partellus* into the stem of three sorghum cultivars[1]

Cultivar	%Larvae boring into different segments (Mean±s.e.)[2]		
	Basal	Middle	Top
IS 18520	40.0±5.8 Aa[3]	23.3±8.8 Ab	3.3±3.3 Ab
IS 18363	46.6±8.8 Aa	23.3±12.0 Ab	3.3±3.3 Ab
IS 1044	30.0±15.3 Ba	0 Bb	0 Bb

[1] 5-7 weeks old plants.
[2] Means of 3 replicates of 10 larvae each released on the outermost leaf ot the whorl of each plant.
[3] Means in each column followed by a different capital letter, and means in each row followed by a different small letter, are significantly different at P=0.05 (ANOVA/DMRT on arcsine transformation).

than of those that moved to IS 1044 (Table 1). However, a total of 63.1% of the larvae was recovered on both cultivars but 36.9% was lost. On the other hand, when the larvae were released on IS 1044 grown between two rows of the susceptible IS 18363, only 19.8% remained on the resistant plants whereas 25.3% emigrated to the susceptible plants, the difference being not significant (Table 1), but the total recovery was only about 45%. These observations show that even if susceptible plants are near resistant ones, larvae dispersing from the latter cannot all reach the susceptible plants.

Larval feeding in the 1st instar. The 1st instar larvae that settled within a leaf whorl would feed (Fig. 1, step 7) on the leaves and make lesions. The area of these lesions, serving as an index of feeding, was almost equally high for IS 18363 and IS

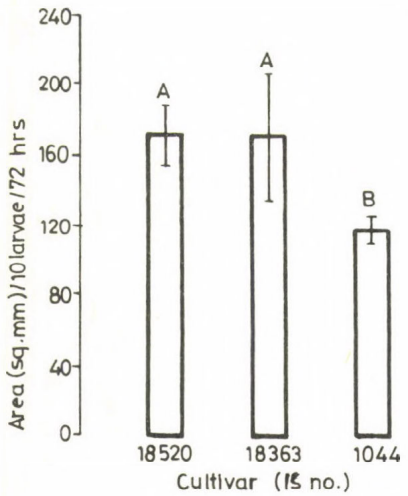

Fig. 4 Consumption of leaves of three sorghum cultivars (3-5 weeks old) by 1st instar *Chilo partellus* larvae. The columns bearing different letters on top differ significantly at P=0.05 (ANOVA/DMRT after log(x+1) transformation).

Table 3 Consumption (in cu mm/larva/72 h) of stem tissues of three sorghum cultivars[1] by 4th instar larvae of *Chilo partellus*

Cultivar	Mean (±s.e.) for each stem segment[2]			Mean (±s.e.) for all segments[3]
	Basal	Middle	Top	
IS 18520	185.7±28.1 a[4]	219.2±34.9 b	172.2±52.9 b	192.4±22.1 b
IS 18363	238.1±20.8 a	373.7±52.1 a	327.8±65.9 a	313.2±31.5 a
IS 1044	80.4±26.4 b	101.3±13.2 c	132.9±34.7 b	104.9±15.2 c

[1] 5-7 weeks old plants.
[2] Means of 5 larvae, each fed on a segment separately.
[3] Means of 15 larvae, each fed on a stem segment separately.
[4] Means in each column followed by different letter are significantly different at P=0.05 (ANOVA/DMRT on log transformed data).

18520 but about 25% less for IS 1044 (Fig. 4). Reduced feeding on IS 1044 would also contribute to its resistance.

Larval arrest and attraction in 4th instar. When the larvae feeding within a leaf whorl develop to late 3rd or early 4th instar, they move out of that feeding site and may move out of that plant, or bore into its stem for further feeding (Fig. 1, steps 9-11). The percentage of the 4th instar larvae that bored into the stem of the same plant was quite high for IS 18363 and IS 18520 but quite low, about 50% less, for the resistant IS 1044 (Fig. 5).

However, the percentage of larvae arriving on single plants was quite low, reflecting a lower attractancy of the cultivars for the 4th instar (Fig. 5) than the 1st (Fig. 3). Nevertheless, the attraction of the resistant IS 1044 was even lower than that of

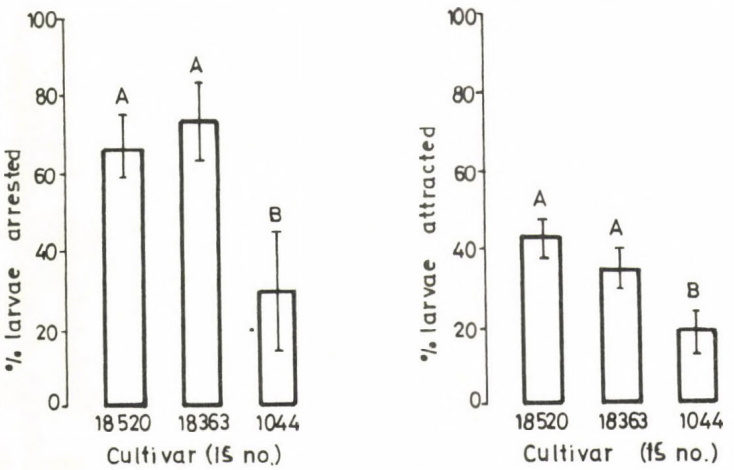

Fig. 5 Arrest and attraction of 4th instar larvae of *C. partellus* by three sorghum cultivars (5-7 weeks old). The columns bearing different letters on top for test series differ significantly from one another at P=0.05 (ANOVA/DMRT after arcsine transformation).

the other cultivar (Fig. 5). This would affect their infestation by dispersing 4th instar larvae.

Of the larvae that were arrested on a plant, the majority bored into the basal region of the stem (Fig. 1, step 13 and Table 2). Even here, the percentage of larvae boring into IS 1044 stem was significantly less than in the other cultivars, thereby further reducing infestation on the resistant cultivar.

Larval feeding on stem in 4th instar. The consumption of stem tissues by the 4th instar larvae also differed among basal, middle and top segments (Table 3). On the whole, the consumption of IS 18363 was higher than that of IS 18520, and that of IS 1044 lowest (about one-third of that for IS 18363) which would further reduce the colonization level on that cultivar.

Relative contribution of different behavioural responses to resistance or susceptibility of sorghum cultivars. Our observations show, therefore, that certain responses of *C. partellus* to a sorghum cultivar may be higher in one cultivar than in another. Low responses will tend to reduce, and high responses to raise, the level of colonization of a particular cultivar. The differences in the colonization of various sorghum cultivars, and hence their susceptibility or resistance to the stem borer, are determined by the relative contributions of all the responses of the insect. Such relative contributions can be considered on the basis of a series of ratios between the response to a cultivar and the response to a standard reference (or check) cultivar. IS 18520 is used here as the check since it is tolerant to the borer and is grown widely in the study area, where it is known as 'Serena'.

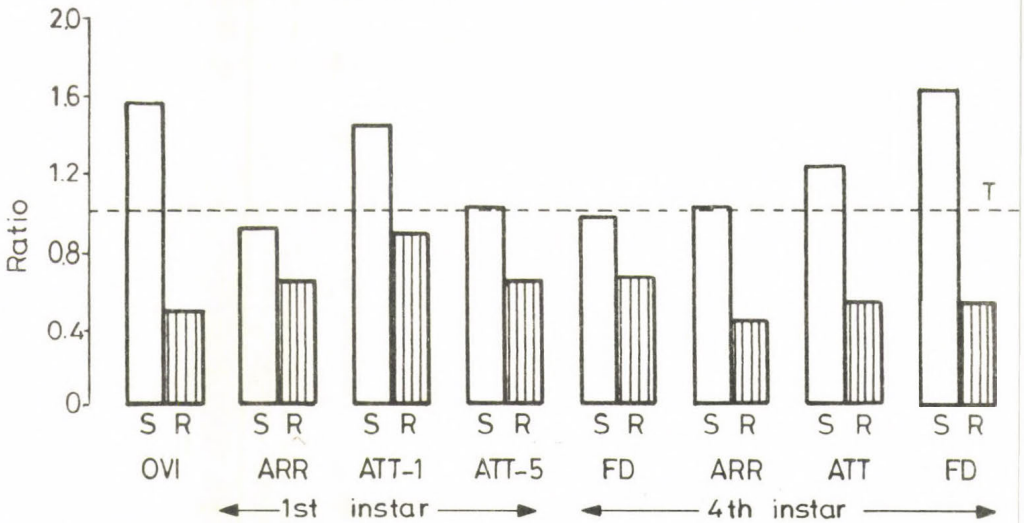

Fig. 6 Ratios between the responses of *C. partellus* to the susceptible (S) or resistant (R) cultivar and those to the tolerant (T) check cultivar IS 18520.
ARR: larval arrest; ATT1: larval attraction to single plants; ATT5: larval attraction to grouped plants in 5-row plots; FD: larval feeding on leaves by 1st instar and on stem by 4th instar; OVI: oviposition. Dotted line shows the level of response to the tolerant check cultivar.

The ratios representing relative levels of different responses of the stem borer are shown Fig. 6 and can be categorised into 5 grades: < 0.4 (very low), 0.4–0.8 (low), 0.8–1.2 (medium), 1.2–1.6 (high), >1.6 (very high). The values of the ratios for the check IS 18520 are set at 1.0 (medium grade). For IS 18363, 3 responses are in the medium grade (Fig. 6) i.e. 1st instar larval arrest, feeding and 4th instar arrest. The remaining responses are in the high grade and thus contribute to its greater susceptibility to *C. partellus* than the check.

On the other hand, all the responses of the stem borer to IS 1044, except 1st instar attraction to single plants, are low. Two of these, i.e. oviposition and 4th instar arrest, are reduced to almost one-half of the check and will contribute most to its resistance. The remaining responses are reduced by about one-third compared to the check and will contribute rather less to the resistance of this cultivar.

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REFERENCES

Bernays, E. A., R. F. Chapman and S. Woodhead (1983): Behaviour of newly hatched larvae of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) associated with their establishment in the host plant, sorghum. *Bull. entomol. Res. 73,* 75-83.

Chapman, R. F., S. Woodhead and E. A. Bernays (1983): Survival and dispersal of young larvae of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in two cultivars of sorghum. *Bull entomol. Res. 73,* 65-74.

Dabrowski, Z. T. and E. L. Kidiavai (1983): Resistance of some sorghum lines to the spotted stalk-borer *Chilo partellus* under western Kenya conditions. *Insect Sci. Appl. 4,* 119-126.

Kumar, H. and K. N. Saxena (1985): Oviposition by *Chilo partellus* (Swinhoe) in relation to its mating, diurnal cycle and certain non-plant surfaces. *Appl. Entomol. Zool. 20,* 218-221.

Lal, G. and J. C. Pant (1980): Laboratory and field testing for resistance in maize and sorghum varieties to *Chilo partellus* (Swinhoe). *Ind. J. Entomol. 42,* 606-610.

Ochieng, R. S., F. O. Onyango and M. D. O. Bungu (1985): Improvement of techniques for mass culture of *Chilo partellus* (Swinhoe). *Insect Sci. Appl. 6,* 425-428.

Roome, R. E. (1980): Dispersal of newly hatched *Chilo partellus* (Swinhoe) larvae from sorghum cultivars. *Z. Ang. Entomol. 90,* 174-180.

Saxena, K. N. (1985): Behavioural basis of plant resistance or susceptibility to insects. *Insect Sci. Appl. 6,* 303-313.

Saxena, K. N. (1987): Ovipositional responses of the stem borer *Chilo partellus* (Swinhoe) to certain sorghum cultivars in relation to their resistance or susceptibility. In: V. Labeyrie, G. Fabres and D. Lachaise. (eds), *Insect-Plants*, Dr. W. Junk Publ., Dordrecht. 313-318.

Singh, B. U. and B. S. Rana. (1984): Influence of varietal resistance on oviposition and larval development of stalk-borer *Chilo partellus* (Swin.) and its relationship to field resistance in sorghum. *Insect Sci. Appl. 5,* 287-296.

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VOLATILE VERSUS NON-VOLATILE ALLELOCHEMICALS IN BEAN PLANT-INSECT INTERACTIONS

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ABSTRACT

Both volatile and non-volatile chemicals contribute to the parameters which determine plant-insect interactions. However, the relative roles of such compounds in determining the botanical range of an insect's hosts have received limited study. Our investigations of some food legumes in these regards have shown that the first line of chemical defense for the highly insect-resistant PI 227687 soybean involves volatiles, and especially two repellents, 3-tetradecene and 1-dodecene. These volatiles make PI 227687 highly resistant to both the extremely polyphagous cabbage looper (CL) as well as the oligophagous Mexican bean beetle (MBB). Relatively non-volatile phenylpropanoid metabolites, and especially flavonoid antifeedants and antibiotics, in this PI constitute a secondary level of chemical defense. A less insect-resistant soybean, commercial cultivar "Davis", proved attractive to both insects but retained a phenylpropanoid-based chemical defense which is lethal to oligophagous MBB larvae. A highly preferred host, lima bean, was neither attractive nor repulsive to either insect, CL or MBB. Thus, distinct defense chemistries are involved in making a legume resistant to a polyphagous versus an oligophagous insect.

Key words: phytochemicals, *Glycine max, Phaseolus lunatus, Trichoplusia ni, Epilachna varivestis*, insect behavior, attractant, repellent, tetradecene, dodecene, plant rejection, acceptance.

INTRODUCTION

The relative roles of volatile versus non-volatile chemicals in plant-insect interactions have remained unclear. However, some insects are known to distinguish among host and non-host plants based primarily on chemicals perceived at a distance from the source (Gilbert et al., 1967; Feeny et al., 1970; Kamm and Buttery, 1983; Khan et al., 1987; Liu et al., 1988, 1989). Others apparently chemically recognize a plant only after arrival on it (Kennedy, 1977). The relative importance of chemical repellents, deterrents and inhibitors versus attractants, arrestants and feeding excitants in plant-insect interactions also is not clear, though Jermy (1966) and Gilbert and Norris (1968) placed emphasis on deterrents and repellents.

The roles of volatiles versus non-volatiles in specifically influencing the botanical host range of given insects have received little study. Results of such investigations in some food legumes regarding the oligophagous Mexican bean beetle (*Epilachna*

varivestis Mulsant) and the polyphagous cabbage looper, *Trichoplusia ni* (Hübner), are presented in this paper.

EXPERIMENTAL ASPECTS

Plants. The test soybeans (*Glycine max* (L.) Merr.) were plant introduction (PI) 227687, reported as relatively resistant to the extremely polyphagous cabbage looper (CL) (*T. ni*) (Luedders and Dickerson, 1977; Khan et al., 1986a, 1986b) and the oligophagous Mexican bean beetle (MBB) (*E. varivestis*) (Van Duyn et al., 1971; Chiang et al., 1986; Rufener II et al., 1986); and "Davis", a commercial cultivar shown to be more susceptible than PI 227687 to CL (Khan et al., 1986a, 1986b) and MBB (Chiang et al., 1986) feeding. The lima bean (*Phaseolus limensis* Macfady) variety was "Henderson", one of the more preferred hosts of CL (Shorey et al., 1962) and MBB (Flander, 1984). The highly uniform growing conditions for the plants in the University of Wisconsin-Madison Biotron and in the U.W.-Mad. greenhouses were detailed by Chiang et al. (1986, 1987) and Liu et al. (1988, 1989).

Trapping of volatiles. Fully expanded trifoliolate leaves (100 g) from soybean or lima bean (fresh or frozen) were placed in a modified 1000 ml erlenmeyer-flask chamber with ground-glass openings-fittings. A Tenax trap consisting of a Pyrex tube packed with 0.17 g Tenax-GC as a 0.5 cm diam. by 9 cm long column was used to collect the volatiles as they were sucked through the column in previously cleaned and dried air. Further details of such collections are given by Liu et al. (1988, 1989).

Extraction and fractionation of plant non-volatiles. Individual leaves that had been stored in methanol at -20°C in darkness were homogenized for 3 min in 50 ml of 100% methanol using an Omni-mixer. The resultant homogenate was filtered through Whatman No. 1 filter paper. The total filtrate per sample was rotaevaporated to dryness in a 50 ml pear-shaped flask in a 50°C water bath. Extractables were redissolved in 50 ml of a mixture of double-distilled (dd) water, ethyl acetate and ethanol (25:30:5); and then partitioned four times in an excess of ethyl acetate, dd water and ethanol (50:1:5) in a separatory funnel. Combined ethyl acetate fractions per sample were rotaevaporated to dryness. Such dried extractables were redissolved in 10 ml ethyl acetate. This was dried under a nitrogen stream in a preweighed sealable vial, sealed and stored at -20°C in a desiccator until used for HPLC. For other details see Neupane and Norris (1990).

Bioassays of plant volatiles. Behavioral responses of CL and MBB female adults to plant volatiles were studied in an open-ended horizontal glass-tube arena (Liu et al., 1988, 1989). Each open end was covered uniformly with either a treated or control filter-paper disk. The assay tube was divided into quadrants. Each arena had a centered side wall opening for introduction of the assay insect. A single female adult was thus placed in the center of the assay arena. With a stopwatch, insect orientation and movement were recorded in seconds, according to quadrant.

Bioassay of plant non-volatiles. To assay chemicals in intact leaves, disks (12 mm diam.) were cut from each test leaf with a No. 6 cork borer. Two leaf disks, one from

Table 1 Responses of CL and MBB female adults to volatiles from soybean and Lima bean leavesa

		CL[c]		
	Treatment[b]	c side	t side	t - c[e]
I.	PI 227687	72.3	27.7	–44.6**
II.	Davis	21.2	78.8	+57.6**
III.	Henderson	51.3	48.7	-2.6 NS
IV.	Solvent control	50.8	49.2	-1.6 NS
V.	Filter-paper control	51.8	48.2	-3.6 NS
		MBB[d]		
	Treatment[b]	c side	t side	t - c[e]
I.	PI 227687	60.9	39.1	–21.8*
II.	Davis	39.7	60.3	+20.6*
III.	Henderson	49.4	50.6	+1.2 NS
IV.	Solvent control	48.9	51.1	+2.2 NS
V.	Filter-paper control	52.5	47.5	–5.0 NS

[a]Data are the mean times, as percentages, that insects spent in each half (side) (i.e., c = control and t = treated) of the assay arena.
[b]Treatments consisted of 40 μl of hexane extractables of plant volatiles obtained by Tenax trapping plus 50 μl of white oil (I-III), solvent control was 40 μl of hexane plus 50 μl of white oil (IV), and V was only filter paper.
[c]In each replication one female adult *T. ni* was assayed for 300 s (5 min).
[d]In each replication one female *E. varivestis* was assayed for 1800 s (30 min).
[e]Differences between means followed by a single asterisk are significantly different at P = 0.05 level (t-test); double asterisks, P = 0.01 level; NS, not significant.

a candidate non-host plant and the other from the host control (lima bean), were each secured with a 2.5 cm insect pin opposite one another, 2 mm apart, in the center of a petri-dish arena. Each petri dish (9 cm diam.) contained a 3 mm bed of paraffin covered by a clean piece of Whatman No. 1 filter paper. One third-instar CL larva (previously starved for 2 h) was placed into the prepared petri-dish arena and allowed to make a free choice in feeding at 27±2°C and 65±10% RH. Each assay was run 6-8 h. See Chiang et al. (1986) for modifications of this assay for adult or larval MBB feeding tests.

To assay extracted non-volatiles or pure compounds, such chemicals were applied to disks (see above) cut from leaves of the preferred host "Henderson" lima bean grown in the greenhouse at 27±5°C under natural light. The abaxial surface of one of each pair of leaf disks was treated with 20 μl of the given extractables or pure chemical; and the other, with just 20 ml of solvent as a control. Other aspects of the bioassays were given above.

The area eaten (cm^2) in a leaf disk by the insect was measured using an automatic area meter (Model LI-3100, LI-COR, Lincoln, NE., U.S.A.)

Purification and identification of plant volatiles. Qualitative and quantitative analyses of the Tenax-GC trapped plant volatiles were conducted by HPLC using an Ultrasphere (25 cm long, 4.6 mm diam.) column of 5 μm diam. silica particles and a variable-wavelength UV spectrophotometric detector. Analyses were also per-

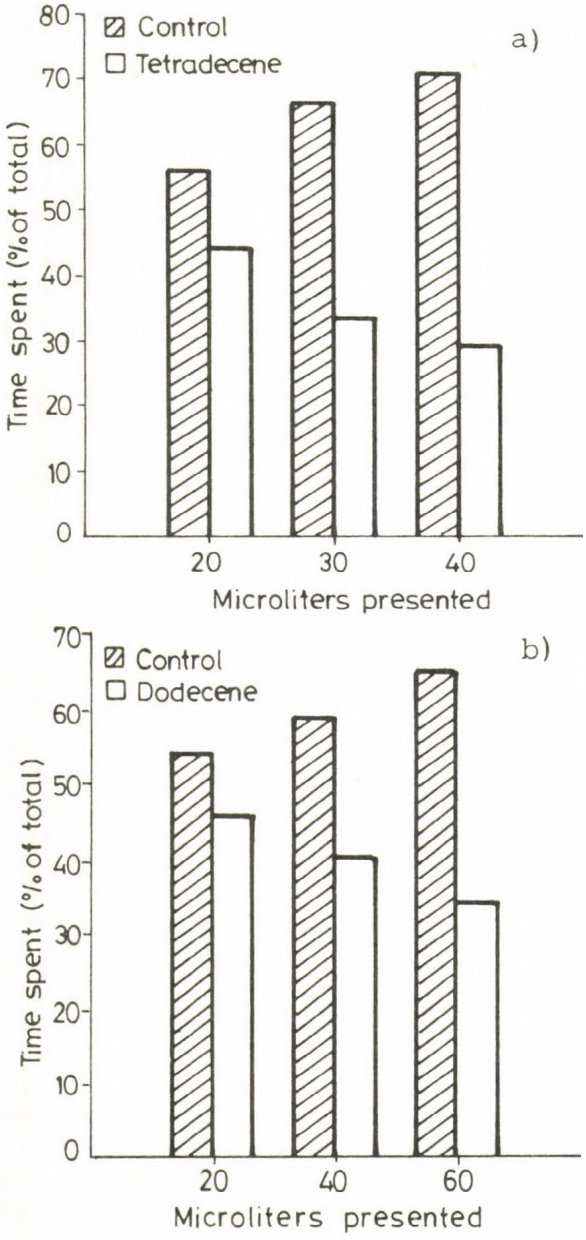

Fig. 1 Behavioral effects of different dosages of commercially obtained (a) tetradecene (0.05%) or (b) dodecene (0.015%) on cabbage looper adult females. All dosages of both compounds, except 20 μl dodecene, gave differences significant at $P<0.05$ or better (t-test).

formed using capillary GLC alone, and capillary GLC-MS. See Liu et al. (1988, 1989) for further analytical details.

Purification and identification of plant non-volatiles. Qualitative analyses of plant non-volatiles were especially accomplished using TLC on silica plates. Details of procedures are given by Khan et al. (1986a) and Sharma and Norris (1990). Qualitative and quantitative determinations were made by HPLC (Sharma and Norris, 1990; Neupane and Norris, 1990). Chemical structures were established based especially on capillary GLC-MS (Sharma and Norris, 1990).

RESULTS

Plant volatiles. Hexane extractables eluted from Tenac-GC trappings from the highly insect-resistant PI 227687 soybean were significantly ($P<0.05$ or 0.01) (Table 1) repellent to both gravid *T. ni* adult females and sexually mature *E. varivestis* adult females. Such volatiles from the less insect-resistant commercial soybean cultivar "Davis" were highly ($P<0.01$) attractive to both CL and MBB adults (Table 1). Such odors from the highly preferred host "Henderson" lima bean were "behaviorally neutral" to both adult female insects (Table 1).

The major components which make PI 227687 volatiles repellent to CL and MBB are 3-tetradecene and 1-dodecene (Fig. 1). The attractive "Davis" volatiles lack the two repellents found in PI 227687, and have 4-hexen-1-ol acetate, 2,2-dimethylhexanal and 2-hexenal as major components. The "behaviorally neutral" "Henderson" lima bean volatiles proved more complex both qualitatively and quantitatively than those of either soybean (Liu et al., 1989).

Plant non-volatiles. The identified flavonoids, daidzein, coumestrol and glyceollins; and one purified but yet unidentified flavonoid (TLC Rf 0.19); Sharma and Norris, 1990) are among the antifeedants and antibiotics in soybean plants against *T. ni* larvae (Khan et al., 1986a; Sharma and Norris, 1990). Extractables which include such flavonoids are also antifeedants and antibiotics to *E. varivestis* larvae. The amounts of such flavonoids in soybean plants are alterable by certain environmental stresses (e.g., insect herbivory or some classical sulfhydryl reagents; Neupane and Norris, 1990).

DISCUSSION

Presented results reveal parameters within a plant species, *G. max*, for chemically distinguishing an evolved resistance to an extremely polyphagous insect, *T. ni*, from such to an oligophagous one, *E. varivestis*. Human creation of the genome which yields the commercial soybean cultivar "Davis" clearly indicates that the genetic regulation of volatile-based chemical resistance to insects in *G. max* is separate from that of relatively non-volatile-based (e.g., flavonoid-based) chemical resistance to insects in this plant species. This separateness allowed plant breeders to make,

apparently unknowingly, "Davis" attractive to adult female *E. varivestis*, but deadly to feeding larvae of this species. This plant "death trap" for an economically important insect species reveals some of the potential for bioengineering plant resistance based on phytochemistry.

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REFERENCES

Chiang, H. S., D. M. Norris, A. Ciepiela, A. Oosterwyk, P. Shapiro and M. Jackson (1986): Comparative constitutive resistance in soybean lines to Mexican bean beetle. *Entomol. exp. appl. 42*, 19-26.

Chiang, H. S., D. M. Norris, A. Ciepiela, A. Oosterwyk, P. Shapiro and M. Jackson (1987): Inducible versus constitutive PI 227687 soybean resistance to Mexican bean beetle, *Epilachna varivestis. J. Chem. Ecol. 13*, 741-749.

Feeny, P., K. L. Paauwe and N. J. Demong (1970): Flea beetles and mustard oils: Host plant specificity of *Phyllotreta cruciferae* and *P. striola* adults (Coleoptera: Chrysomelidae). *Ann. Entomol. Soc. Amer. 63*, 832-841.

Flander, R. V. (1984): Comparisons of bean varieties currently being used to culture the Mexican bean beetle (Coleoptera: Coccinellidae). *Environ. Entomol. 13*, 995-999.

Gilbert, B. L., J. E. Baker and D. M. Norris (1967): Juglone (5-hydroxy-1,4-naphthoquinone) from *Carya ovata*, a deterrent to feeding by *Scolytus multistriatus. J. Insect Physiol. 13*, 1453-1459.

Gilbert, B. L. and D. M. Norris (1968): A chemical basis for bark beetle (*Scolytus*) distinction between host and non-host trees. *J. Insect Physiol. 14*, 1063-1068.

Jermy, T. (1966): Feeding inhibitors and food preference in chewing phytophagous insects. *Entomol. exp. appl. 9*, 1-12.

Kamm, J. A. and R. G. Buttery (1983): Response of the alfalfa seed chalcid, *Bruchophagus roddi*, to alfalfa volatiles. *Entomol. exp. appl. 33*, 129-134.

Kennedy, J. S. (1977): Olfactory responses to distant plants and other odor sources. In: H. H. Shorey and J. J. McKelvey (eds), *Chemical Control of Insect Behavior, Theory and Application*. John Wiley and Sons, Inc., New York.

Khan, Z. R., D. M. Norris, H. S. Chiang, N. E Weiss, and A. S. Oosterwyk (1986a): Light-induced susceptibility in soybean to cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae). *Environ. Entomol. 15*, 803-808.

Khan, Z. R., J. T. Ward and D. M. Norris (1986b): Role of trichomes in soybean resistance to cabbage looper, *Trichoplusia ni. Entomol. exp. appl. 42*, 107-117.

Khan, Z. R., A. Ciepiela and D. M. Norris (1987): Behavioral and physiological responses of cabbage looper, *Trichoplusia ni* (Hübner), to steam distillates from resistant versus susceptible soybean plants. *J. Chem. Ecol. 13*, 1903-1915.

Liu, S.-H., D. M. Norris and E. Marti (1988): Behavioral responses of female adult *Trichoplusia ni* to volatiles from soybeans versus a preferred host, lima bean. *Entomol. exp. appl. 49*, 99-109.

Liu, S.-H., D. M. Norris and P. Lyne (1989): Volatiles from the foliage of soybean, *Glycine max*, and lima bean, *Phaseolus lunatus*: Their behavioral effects on the insects *Trichoplusia ni* and *Epilachna varivestis*. *J. Agr. Food Chem. 37*, 496-501.

Luedders, V. D. and W. A. Dickerson (1977): Resistance of selected soybean genotypes and segregation populations to cabbage looper feeding. *Crop Sci. 17*, 395-397.

Neupane, F. P. and D. M. Norris (1990): Iodoacetic acid alteration of soybean resistance to the cabbage looper (Lepidoptera: Noctuidae). *Environ. Entomol.* (in press).

Rufener II, G. K., R. B. Hammond, R. L. Cooper and S. K. St. Martin (1986): Mexican bean beetle (Coleoptera: Coccinellidae) development on resistant and susceptible soybean lines in the laboratory and relationship to field selection. *J. Econ. Entomol. 79*, 1354-1358.

Sharma, H. C. and D. M. Norris (1990): Antifeedant and antibiotic effects of flavonoids in insect-resistant PI 227687 soybean to cabbage looper, *Trichoplusia ni*. *J. Chem. Ecol.* (in press).

Shorey, H. H., L. A. Andres and R. L. Hale, Jr. (1962): The biology of *Trichoplusia ni* (Lepidoptera: Noctuidae). I. Life history and behavior. *Ann. Entomol. Soc. Amer. 55*, 591-597.

Van Duyn, J. W., S. G. Turnipseed and J. D. Maxwell (1971): Resistance in soybeans to the Mexican bean beetle. I. Sources of resistance. *Crop Sci. 11*, 572-573.

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OLFACTORY RECOGNITION OF PLANTS BY INSECT PESTS: *DROSOPHILA* AS A MODEL SYSTEM

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ABSTRACT

Drosophila melanogaster is considered as a model system for study of insect olfaction. As an illustration of its potential value in investigating olfactory function, a simple method of isolating mutants defective in olfactory behavior is described, and a description of one particular mutant, *ota1*, is presented. This mutant is defective not only in olfactory response but also in the electrophysiology of its visual system. Genetic analysis indicates that the two abnormalities are likely to be caused by the same mutation. This relationship between olfactory and visual response suggests a powerful approach to further investigation of olfaction in this insect.

Key words: Drosophila melanogaster, genetics, olfactory mutants.

INTRODUCTION

An understanding of the mechanisms by which insects recognize their plant hosts may be useful in designing new means in pest control. Olfaction is known to be central to the process of host recognition for many insects, yet little is known about the molecular mechanisms underlying olfactory function. A detailed understanding of the means by which plant compounds stimulate signals in the insect olfactory system and the means by which these signals are processed to generate behavioral responses might suggest improved methods for controlling harmful insects.

DROSOPHILA AS A MODEL SYSTEM FOR STUDY OF OLFACTION

Drosophila is a significant pest for a variety of crops (Fitz-Earle and Holm, 1983), primarily in that it acts as a vector for undesirable microorganisms. It exhibits a strong olfactory response to a variety of volatile chemicals at both the larval and adult stages (Siddiqi, 1987; Monte et al., 1989), and it exhibits associative learning when presented with olfactory and other stimuli (Quinn et al., 1974). The anatomy of its olfactory sytem has been characterised in some detail (e.g. Venkatesh and Singh, 1984; Stocker et al., 1983), and physiological responses can be characterized

either by electroantennogram or by single unit recording (Venard and Pichon, 1981; Siddiqi, 1984).

Perhaps the greatest advantage of *Drosophila* as a model system for study of insect olfactory function, however, is the feasibility of performing genetic and molecular analysis. The small size, short life cycle, and ease of culturing *Drosophila melanogaster* have made it a favored organism for genetic analysis, and over the past 75 years an enormous number of mutant strains have been isolated and constructed (Lindsley and Grell, 1968). These include not only mutants displaying visible defects in external morphology, but also mutants exhibiting a wide variety of behavioral and physiological defects (Hall, 1982).

The richness of *Drosophila* genetics, along with its small genome size and its easily visible polytene chromosomes, have made *Drosophila* a convenient organism to study at the molecular level. Techniques now exist to allow cloning of essentially any gene which can be identified by mutation (Pirrotta, 1986). The large number of genes which have been cloned and characterized at the molecular level now includes genes encoding receptors, components of second messenger pathways, and ion channels.

The power of *Drosophila* genetics and molecular biology in analysing sensory system function has already been amply demonstrated by the case of the visual system. Benzer (1967) designed an elegant test of visual behavior and used it to isolate a set of mutants defective in visual function. Among a variety of mutants isolated in this and other assays of visual behavior, a number have been found to be defective in visual physiology, and several of these genes defined by these mutations have been

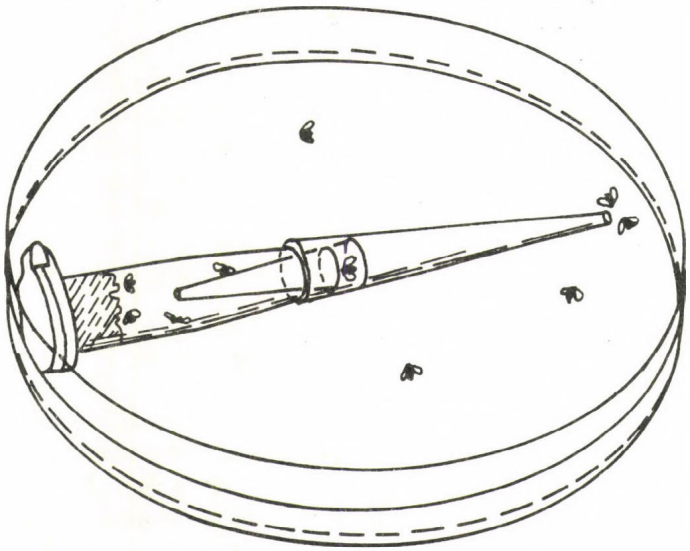

Fig. 1 The trap assay. Ten flies are placed in the Petri dish along with a trap containing an olfactory attractant. The trap is constructed from a microfuge tube and two micropipette tips. The number of flies in the trap in counted as a function of time. (From Woodard et al., in press).

molecularly cloned. An example of such mutant is *norpA*, which is behaviorally blind and which fails to undergo normal retinal depolarization (Pak, 1979). The convenient genetic and molecular technology available for investigation of *Drosophila* allowed the cloning of the *norpA* gene (Bloomquist et al., 1988). Nucleotide sequence analysis showed it to be homologous to phospholipase C, thereby providing strong evidence that phototransduction operates by means of the IP_3 second messenger system, of which phospholipase C is a key component (Bloomquist et al., 1988; see also Yoshioka, 1985).

The success of using behavioral genetics in analysing the *Drosophila* visual system has encouraged others to use it for investigation of the olfactory system. Among the earliest advances in this direction were those of Rodrigues and Siddiqi (1978), who isolated a number of olfactory mutants using a Y-maze assay. This assay, while attractive in a number of important ways, also suffers some practical and theoretical difficulties as a means of isolating mutants of *Drosophila* defective in olfactory attraction response. We have therefore developed another assay, an olfactory trap assay, designed to be convenient for genetic analysis (Woodard et al., in press).

A SIMPLE MEASURE OF OLFACTORY RESPONSE

The olfactory trap assay is compact and simple. It consists of a Petri dish, a microfuge tube, and two plastic disposable micropipette tips (Fig. 1). An olfactory attractant, such as *Drosophila* culture medium or a pure chemical such as ethyl acetate, is placed in the microfuge tube. The end of the microfuge tube is then cut off, and a truncated pipette tip is placed with its small end inside the tube. A second truncated pipette is placed over the first, with the small end pointed in the opposite direction. This trap is then placed inside an agarose Petri dish along with 10 flies. In order to reach the attractant, the flies must crawl through the pipette tips. The pipette tip with the small end outside makes it difficult for the flies to enter the trap; the pipette tip with the small end inside makes it difficult for the flies, once entered to exit. Response is scored by counting the number of flies in the trap as function of time. The number of trapped flies is a good measure of the entry rate, since the exit rate is very low.

A series of experiments indicated that the response is in fact driven by olfaction (Woodard et al., in press). First, if no olfactory stimulus is placed in the traps, response is very low. (The dimensions and geometry of the trap were designed in part to make it unlikely that flies would enter the trap at random, in the absence of olfactory input; perhaps because of the difficulty of the task, flies do not respond quickly, and the assay is usually carried out over a period of approximately 60h.) Second, flies give similar response in light and in the dark, indicating that the response does not depend on visual input. Finally, following surgical removal of the third antennal segments, which are the primary olfactory organs of the fly (Barrows, 1907; Venard and Pichon, 1981), flies fail to respond.

A MUTANT DEFECTIVE IN OLFACTORY RESPONSE

A screen for mutants defective in response to the attractant ethyl acetate yielded a set of six mutants, called the *ota* mutants (*ota* = *olfactory trap abnormal*). Among these is the mutant *ota1*, which is X-linked and which was induced by the chemical mutagen ethyl methane sulphonate (Fig. 2a). *A priori*, all that is known about *ota* mutants such as *ota1* is that they fail to respond in the trap assay – they could be defective at the sensory level, or other levels. One means of characterizing such mutants further is physiological analysis.

A detailed analysis of antennal physiology of *ota1* mutants is currently in progress; an analysis of visual system physiology has been completed and shows a clear defect

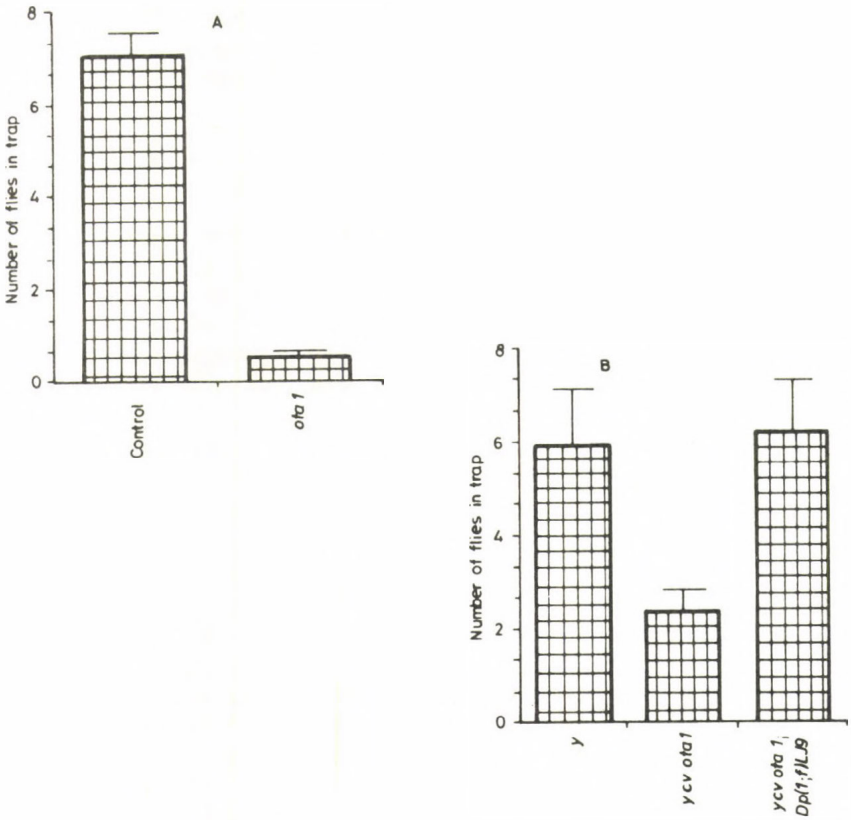

Fig. 2 (A) Trap assay responses of *ota1* and Canton-S control. n=20 groups of 10 tested flies for *ota1*; n=77 groups for control. Values indicate the mean (±SEM) number entering the trap after 60h in response to 0.5% ethyl acetate. (B) Response of *y cv ota1* flies (n=10), their sibling *y cv ota1* flies carrying *Dp(1;f)LJ9* (n=10), and control *y* flies (n=10). The *cv* marker does not affect response.

(Fig. 3a,b). *ota1* exhibited defective retinal depolarization in all of 30 individuals examined. The characteristic "on" and "off" transients, which arise in wild-type from post-synaptic activity in the lamina (Goldsmith and Bernard, 1974; Coombe, 1986), are also absent in *ota1*. Thus *ota1* is defective in the physiology of at least one sensory system. To asses the specificity of the *ota1* physiological defect, a test of a giant fiber system physiology (Wyman et al., 1984) was also conducted. This test, which requires normal function of at least four defined types of neurons, normal transmission at five synapses, and normal electrical response of two muscle types, revealed no ab-

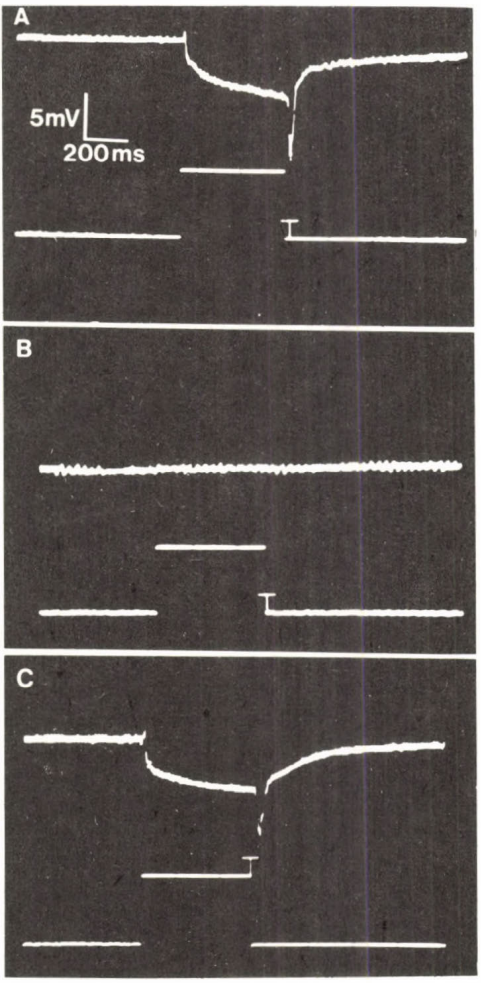

Fig. 3 Electroretinogram (ERG) recording of: (A) wild-type. Note sustained receptor potential and "on" and "off" transients; (B) *ota1*, marked with *y* and *cv*; (C) *y cv ota1* carrying *Dp(1;f)LJ9*. An artifact of the triggering system has been partially removed from the photographs.

normality in *ota1*. These results argue that *ota1* does not contain a very general neural or motor defect; rather, they argue that the mutant contains a more specific defect.

GENETIC ANALYSIS OF THE *ota1* MUTANT

The *ota1* mutant thus displays a defect in olfactory behavior as well as a defect in visual system physiology. Are these two effects a consequence of a single mutation, or has *ota1* coincidentally acquired two independent abnormalities? Genetic mappings allows localization of the mutation causing each effect; if the two effects can be mapped to the same chromosomal position, then they are likely to be caused by the same mutation.

Genetic mapping by recombination analysis allowed the visual defect to be localized approximately to position 43 on the meiotic recombination map of the X chromosome (Fig. 4). This position is within region 12 of the cytogenetic map of the X chromosome, a map based on the pattern of bands seen in the giant polytene chromosomes of the larval salivary gland. *Dp(1;f)LJ9*, a small segment of the normal, wild-type X chromosome extending from a position within region 12(12A6-10) on one end to a position within the adjacent region 13(13A2-5) on the other hand, was crossed into the *ota1* stock, and *ota1* males containing this small segment were found to have normal visual physiology (Fig. 3). Not only does *Dp(1;f)LJ9* restore normal visual physiology, but, as shown in a separate experiment, it also restores normal olfactory behavior (Fig. 2b). We conclude that the mutations responsible for both effects are contained within the corresponding segment of the *ota1* X chromosome. Since this segment is small, it is unlikely to contain two independent mutations; it is much more likely that both effects are due to a single mutation.

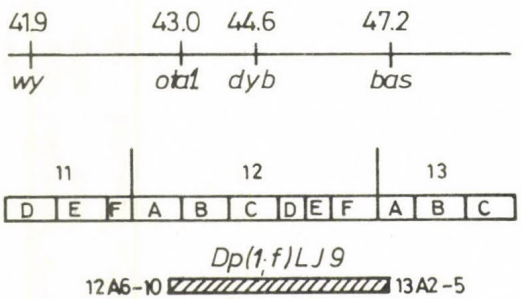

Fig. 4 Recombinational map (top line) and cytogenetic map of the X chromosome showing approximate location of *ota1 and Dp(1;f)LJ9*.

CONCLUSIONS

The genetic analysis described above provides strong evidence that the *ota1* gene is required for both visual and olfactory response. The finding of commonality between the two systems offers considerable promise for the dissection of the olfactory pathway, in light of the large accumulation of information concerning the *Drosophila* visual system. For example, testing of defined visual system mutants in olfactory assays may implicate well-defined genes in olfactory function. Since many of these genes have been characterized not only genetically, but also have been cloned and characterized at the molecular level, their implication in olfactory function might readily provide a great deal of information about olfaction.

REFERENCES

Barrows, W. (1907): The reactions of the pomace fly, *Drosophila ampelophila* Loew, to odorous substances. *J. Exp. Zool. 4,* 515-537.

Benzer, S. (1967): Behavioral mutants of *Drosophila* isolated by countercurrent distribution. *Proc. Nat. Acad. Sci. 58*, 1112-1119.

Bloomquist, B. T., R. D. Shortridge, S. Schewly, M. Perdew, C. Montell, H. Staller, G. Rubin and W. L. Pak (1988): Isolation of a putative phospholipase C gene of *Drosophila*, norp A, and its role in phototransduction. *Cell 54,* 723-733.

Coombe, P. E. (1986): The large monopolar cells L1 and L2 are responsible for ERG transients in *Drosophila. J. Comp. Physiol. 159,* 655-665.

Fitz-Earle, M. and D. Holm (1983): *Drosophila melanogaster* models for the control of insect pests. In: M. Ashburner, H. Carson, and J. Thompson (eds), *The Genetics and Biology of Drosophila.* Vol.3c. Academic Press, New York. 399-414.

Goldsmith, T. and G. Bernard (1974): The visual system of insects. In: M. Rockstein (ed), *The Physiology of Insects*. Academic Press, New York. 165-272.

Hall, J. C. (1982): Genetics of the nervous system in *Drosophila. Q. Rev. Biophys. 15,* 223-479.

Jallon, J. (1984): A few chemical words exchanged by *Drosophila* during courtship and mating. *Behav. Genet. 14,* 441-477.

Lindsley, D. L. and E. H. Grell (1968): *Genetic Variations of Drosophila melanogaster.* Carnegie Institution of Washington Publ., No. 627.

Monte, P., C. Woodard, R. Ayer, M. Lilly, H. Sun, and J. Carlson (1989): Characterisation of the larval olfactory response in *Drosophila* and its genetic basis. *Behav. Genet. 19,* 267-283.

Pak, W. (1979): Study of photoreceptor function using *Drosophila* mutants. In: X. Breakefield (ed), *Neurogenetics: Genetic Approaches to the Nervous System.* Elsevier-North Holland, New York. 67-99.

Pirotta, V. (1986): Cloning *Drosophila* genes. In: D. Roberts (ed), *Drosophila a Practical Approach.* IRL Press, Oxford. 83-110.

Quinn, W., W. Harris, and S. Benzer (1974): Conditioned behavior in *Drosophila melanogaster. Proc. Natl. Acad. Sci. 71,* 708-712.

Rodrigues, V. and O. Siddiqi (1978): Genetic analysis of chemosensory pathway. *Proc. Ind. Acad. Sci. 81B,* 147-160.

Siddiqi, O. (1984): Olfactory neurogenetics of *Drosophila* . In: V. Chopra, B. Joshi, R. Sharma and H. Bawal (eds), *Genetics: New Frontiers.* Unipub, Ann Arbor. 245-261.

Siddiqi, O. (1987): Neurogenetics of olfaction in *Drosophila melanogaster. Trends Genet. 3,* 137-142.

Stocker, R., R. Singh, M. Schorderet, and O. Siddiqi (1983): Projection patterns of different types of antennal sensilla in antennal glomeruli of *D. melanogaster. Cell Tissue Res. 232,* 237-284.

Venard, R. and Y. Pichon (1981): Etude electro-antennographique de la réponse périphérique de l'antenne de *Drosophila melanogaster* à des stimulations odorantes. *C. R. Acad. Sc. Paris 293,* 839-842.

Venkatesh, S. and R. Singh (1984): Sensilla on the third antennal segment of *Drosophila melanogaster* Meigen. *Int. J. Insect Morphol. Embriol. 13,* 51-63.

Woodard, C., T. Huang, H. Sun, S. Helfand and J. Carlson: Genetic analysis of olfactory behavior in *Drosophila*: a new screen yields the *ota* mutants. *Genetics,* (in press)

Wyman, R., J. Thomas, L. Salkoff, and D. King (1984): The *Drosophila* giant fiber system. In: R. Eaton (ed), *Neural Mechanisms of Startle Behavior.* Plenum Press, New York. 133-161.

Yoshioka, T., H. Inoue and Y. Hotta (1985): Absence of phosphatidylinositol phosphodiesterase in the head of *Drosophila* visual mutant, *norpA* (*no receptor potential A*). *J. Biochem. 97,* 1251-1254.

ECOLOGY

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THEORIES OF PLANT CHEMICAL DEFENSE: A BRIEF HISTORICAL SURVEY

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ABSTRACT

Three current hypotheses to account for ecological patterns of chemical resistance in plants are outlined and some of the relevant background research is reviewed briefly. Recent models that incorporate the apparency and resource availability hypotheses as axes of a habitat templet have considerable value in predicting overall patterns of defense allocation between habitats. One drawback to such models, however, is the difficulty of incorporating components of apparency that are unrelated to the habitat variables. A possible route towards the formulation of a more general model might be to consider the impact of habitat quality on resistance in terms of its effects on tissue value and apparency.

Key words: Apparency, tissue value, resistance, defense, habitat quality, habitat templet, qualitative resistance, quantitative resistance, mutualism, tannins, life history, resource availability.

INTRODUCTION

A plant trait may be said to confer resistance to a pathogen or herbivorous animal if its presence reduces damage to the plant by deterring attack or by impairing the growth, survival or reproduction of the attacker. Resistance may be "constitutive", based on the presence of resistance traits prior to attack, or "induced" as a result of interaction between a plant and its attackers (Levin, 1971).

The different forms of plant resistance have been reviewed extensively (eg. Levin, 1976; Chapman and Bernays, 1977; Hedin, 1983; Rosenthal and Janzen, 1979; Maxwell and Jennings, 1980). They can be grouped into three categories, namely physical resistance, nutrient deficiency and chemical resistance, each representing a major hurdle faced by insects in their colonization of plants (Southwood, 1972). To these kinds of resistance should be added the association of plants with natural enemies of the organisms that attack them — the "third trophic level" (Price et al., 1980). These predators, parasites and pathogens play a major role in limiting populations of herbivores, especially of invertebrates, and hence in reducing plant damage (Hairston et al., 1960). Their presence may be an essential complement to forms of resistance that reduce herbivore growth rates (Moran and Hamilton, 1980; Damman, 1987). Many plants produce extrafloral nectaries and other traits that enhance the effectiveness of natural enemies (Janzen, 1966; Bentley, 1977) and association with

such enemies appears, in some cases, to have replaced former chemical resistance (Rehr et al., 1973).

Thirty years after Fraenkel (1959) gave new emphasis to the hypothesis that plant secondary compounds evolved as a means of protection through "reciprocal adaptive evolution" between plants and their enemies, the hypothesis remains controversial (eg. Jermy, 1984, 1988; Bernays, 1987; Bernays and Graham, 1988). While some resistance traits, such as thorns and spines and the traits associated with ant mutualists (Janzen, 1966), clearly serve a primarily defensive function in plants, the role of predation pressure in the evolution of chemical resistance can usually be discerned with much less confidence.

Secondary compounds serve many functions in plants (eg. Seigler and Price, 1976; McKey, 1979) and the resistance they confer may sometimes be incidental to these other functions (Bernays and Graham, 1988). Nevertheless there is much circumstantial evidence to support Fraenkel's coevolutionary interpretation of phytochemical diversity (eg. Ehrlich and Raven, 1964; Janzen, 1973; Feeny, 1977, 1990; Rosenthal and Janzen, 1979; Berenbaum, 1983a; Futuyma, 1976, 1983).

Rather than discussing further the roles of predation in the origins and diversification of secondary compounds, this paper rewievs briefly some hypotheses concerning patterns of ecological convergence in the functional categories and amounts of chemical resistance in plants.

QUALITATIVE AND QUANTITATIVE RESISTANCE

Chemical defenses can be broadly grouped into two ecological categories, "qualitative" and "quantitative", on the basis of their modes of action and their vulnerability to biochemical counteradaptation by herbivores (Feeny, 1975). The distinctions between these categories will be illustrated with examples and some historical background.

Blau et al. (1978) demonstrated unambiguously that plant leaves contain particular compounds that are toxic when ingested by insect herbivores that share the same habitat. They showed that allylglucosinolate (sinigrin) from cruciferous plants was lethal at or below natural concentrations to larvae of the black swallowtail butterfly, *Papilio polyxenes*, which normally feed on plants of the Umbelliferae. The compound was not toxic, even at elevated doses, to larvae of the butterfly *Pieris rapae* (Blau et al., 1978), an adapted specialist whose feeding and growth on typical cruciferous food plants is affected primarily by plant nitrogen content rather than by glucosinolates (Slansky and Feeny, 1977). In parallel studies with compounds from umbelliferous plants, Berenbaum (1978, 1981a) showed that xanthotoxin, a linear furanocoumarin was toxic to larvae of the southern armyworm, *Spodoptera eridania*, but innocuous to the umbellifer-feeding larvae of *P. polyxenes*.

I described the resistance provided by compounds such as glucosinolates and furanocoumarins as "qualitative", since their effects can be overcome, probably at trivial cost, by appropriate physiological or biochemical adaptation (Feeny, 1975).

The effectiveness of such "toxins" (Rhoades and Cates, 1976) is assumed to depend largely on their individual chemical uniqueness and on the idiosyncratic historical constraints of ambient herbivores and pathogens that restrict any one species to tolerating or perceiving as non-deterrent (cf. Bernays, 1987; Jermy, 1988) only a fraction of the compounds in its environment. Being mostly small molecules, subject to rapid biochemical turnover (cf. McKey, 1974, 1979), qualitative defense compounds can often be translocated easily among plant tissues and have also been described as "mobile" defenses (Coley et al. 1985).

The young foliage of oak leaves, *Quercus robur*, in England is often attacked heavily by insects of many species, but the mature leaves typically receive little damage. I found that caterpillars of the winter moth, *Operophtera brumata*, one of the spring defoliators, were unable to grow normally or survive when fed late-spring oak leaves, but growth on such leaves was comparable to that on early-spring leaves if the powdered leaves were incorporated in a plain agar diet (Feeny, 1970). This suggested that leaf toughness is an important component of the resistance of older oak leaves. Low nitrogen content was considered to be another likely component of resistance since mature oak leaves contain only half the concentration of nitrogen that is present in young leaves (Feeny, 1970).

I also found that the growth of winter moth larvae on an artificial diet was reduced when tannins, extracted from September oak leaves, were added to the diet (Feeny, 1968). Hydrolyzable tannins are present in oak leaves throughout the season while condensed tannin, absent from the young leaves, becomes the major tannin in mature foliage (Feeny and Bostock, 1968; Feeny, 1970). When casein or nettle-leaf protein was exposed to the purified oak tannins at pH 5.0 (the pH of macerated oak leaves) and the resulting complexes subjected to trypsin digestion at pH 9.2 (the pH of the winter moth midgut), protein digestion was reduced substantially — the effect being greater with condensed than with hydrolyzable tannin (Feeny, 1969). In view of these results, and more general envidence of the ability of tannins to precipitate proteins and inhibit enzymes (Pridham, 1963; Goldstein and Swain, 1965), I suggested that interference with digestion was responsible for reduced growth by larvae on tannin diets and that the declining availability of nitrogen to insects feeding on mature oak leaves is further reduced by the increasing content of tannins (Feeny and Bostock, 1968; Feeny, 1969).

The poor food quality of mature tree leaves for the larvae of leaf-chewing insects has been shown in other studies (reviewed by Scriber and Slansky, 1981; Raupp and Denno, 1983; see also Coley, 1983; Damman, 1987) and seems to represent a fundamental ecological barrier to rapid growth (Scriber and Feeny, 1979). Food quality is reduced in ways that are generalized and dose-dependent in their action, and that cannot be overcome at trivial cost by specific counteradaptation. In addition to toughness and low nitrogen or water content (cf. Scriber, 1977), components of such "quantitative resistance" (Feeny, 1975) may include deficiencies of other nutrients and the presence of intractable substances such as resins and silica (Scriber and Slansky, 1981; McNaughton and Tarrants, 1983). Most of the world's plant foliage remains unavailable for use as human food because of its quantitative rather than

qualitative resistance (Feeny, 1977). The secondary compounds contributing to quantitative resistance are usually not turned over or translocated easily and have thus been described as "immobile" defenses (Coley et al., 1985).

I originally included tannins within the category of quantitative resistance because it seemed that their antidigestive effects would be difficult for herbivores to overcome (Feeny, 1975). However, all attempts to demonstrate interference by tannins with food digestion by insects have so far failed (Fox and Macauley, 1977; Bernays, 1978, 1981; Reese et al., 1982; Berenbaum, 1983b; Martin and Martin, 1984). Tannins may have deterrent effects (Bernays, 1981; Reese et al., 1982), and hydrolyzable tannins can be toxic (Bernays, 1978; Berenbaum, 1983b). Many insects, however, are able to tolerate tannins with no ill effects and hydrolyzable tannins, at least, may even act as phagostimulants (Bernays, 1981). The potential antidigestive properties of tannins appear to be avoided in several ways, including hydrolysis of hydrolyzable tannins in the gut (Bernays and Chamberlain, 1980), maintainance of an alkaline gut pH (Feeny, 1969; Berenbaum, 1980), adsorption and retention of tannins by the peritrophic membrane (Bernays and Chamberlain, 1980), and inhibition of protein-tannin interaction by surfactants in the gut (Martin and Martin, 1984).

It no longer seems likely that the reduced growth of winter moth larvae that I observed on tannin diets (Feeny, 1968) resulted from antidigestive effects. Nor is it probable that the tannins were acting as toxins, since histological examination revaled no passage of tannins through the peritrophic membrane (Feeny, 1970). More likely, the slow growth resulted from inhibition of feeding rate (cf. Reese et al., 1982), a parameter that was unfortunately not measured in my study.

ECOLOGICAL PATTERNS OF PLANT DEFENSE

a) Tissue value hypothesis. Levels of toxins such as glucosinolates and furanocoumarins are by no means uniform throughout the tissues of plants containing them. The highest concentrations are often found in young leaves and in developing fruits and seeds, while older foliage typically contains lower levels of the compounds (eg. McKey, 1979; Berenbaum, 1981b). The drop in leaf concentrations is unlikely to be a simple consequence of dilution during leaf expansion since many of these compounds are known to be in a constant state of biochemical turnover (Seigler and Price, 1976). A more reasonable hypothesis for intraspecific variation in toxin concentration was advanced by McKey (1974) in terms of resource allocation. Since the compounds are believed to be expensive, in terms of energy and nutrients, the amounts present in particular tissues should reflect the value of those tissues to the plant. By "value" is meant the contribution made by those tissues to plant fitness or, put another way, the reduction in plant fitness that would occur if the tissues were lost. Other things being equal, a given biomass of young foliage would be expected to have greater value than the same mass of older foliage that has lower potential for future productivity and that is also relatively deficient in valuable nutrients. Though the higher concentrations of toxins associated with the more valuable, pro-

tein-rich tissues may not be sufficient to prevent attack by adapted specalist insects, they appear to be effective in warding off attack by generalists (eg. Berenbaum, 1981b).

Many factors affect the costs of secondary compounds and hence their patterns of allocation (McKey, 1979). Plants are subject to a variety of physiological constraints that vary with the availability of resources and hence alter the relative costs of different types of defense (Mooney et al., 1983; Bryant et al., 1983). A plant's carbon/nitrogen balance, in particular, influences the relative costs of carbon-based and nitrogen-based defenses. Plants adapted to high light intensities in nutrient-poor habitats, for example, are likely to have a relative surplus of carbon and hence to deploy carbon-based defenses (Bryant et al., 1983). Unless constrained by other factors, plants should maximize the benefits conferred by secondary compounds relative to the costs of reclaiming or replacing them (McKey, 1979). Quantitative resistance traits, for example, are typically immobile and cannot be reclaimed from senescent leaves; they should therefore be allocated to long-lived tissues. By contrast, one would expect plants to allocate easily reclaimable defenses to short-lived tissues (McKey, 1979). It may be no coincidence that qualitative resistance generally consists of such mobile compounds since mobility and avoidance of autotoxicity in the plant may parallel in fundamental biochemical ways the vulnerability of such compounds to physiological counteradaptation by herbivores (McKey, 1979).

b) Apparency hypothesis. To account for the different kinds and levels of defense in crucifers and oak trees, I suggested that allocations to defense in plants might reflect the extent to which the plants typically escape from herbivores (Feeny, 1975). Crucifers and other herbaceous plants, being ephemeral in space and time, might be relatively hard for adapted enemies to find and hence could be expected to survive with low levels of qualitative defense — enough to ward off generalist feeders and non-adapted insects. The size and persistence of oak trees, by contrast, would render them easy for enemies to find; their foliage should therefore be defended by substantial quantitative resistance, effective against most potential enemies (Feeny, 1975). Qualitative resistance might not long survive the prolonged exposure to possible counteradaptation (cf. Southwood, 1972).

Similar arguments for different allocations to defense in relation to successional status were advanced by Cates and Orians (1975), who supported their hypothesis with the results of feeding trials using generalist slugs. Meanwhile, McKey (1974) suggested that a plant's defense allocation should reflect not only the value of a tissue but also its "vulnerability" — the probability that the tissue would be successfully attacked in the absence of chemical defense. He pointed out that the young foliage of trees that flush their leaves synchronously may escape attack through "phenological protection" and hence be able to survive with little chemical defense. More elaborate hypotheses relating chemical defense to plant "apparency" or "predictability and availability" were advanced simultaneously by Feeny (1976, 1983) and Rhoades and Cates (1976), respectively (cf. Fig. 1).

Fundamental to the apparency hypothesis is the trade-off between resistance and escape that is reflected in the life histories of all organisms. Escape may take various

forms, including escape in space, escape in time, escape in numbers, mimicry and crypsis.

A significant practical problem with the apparency hypothesis is the difficulty of measuring apparency. This may require a more sophisticated understanding of the behavior of foraging herbivores than can readily be gained.

c) Resource availability hypothesis. The apparency hypothesis was tested by Coley (1983, 1987) in light gaps within a lowland rain forest in Panama. Saplings of the many different tree species populating such light gaps could be divided into two categories. The "pioneers" or "light-gap specialists" are intolerant of shade; they germinate when the light gap forms, grow rapidly and must reach the canopy ahead of their competitors or perish in the attempt. By contrast, the "persistents" are able to tolerate shade and also exist throughout the closed forest at various stages of growth. It seemed likely that the persistents, by virtue of their slow growth and broader distribution in the forest understory, might be more apparent to herbivores than are the pioneer species and hence should have higher levels of defense in their foliage.

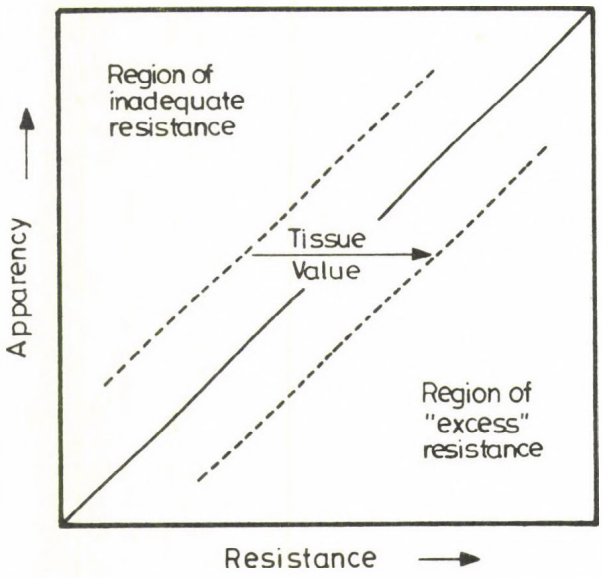

Fig. 1 Graphical representation of the apparency hypothesis (after Feeny 1983). Populations at ecological and evolutionary equilibrium with their enemies are considered to lie within the diagonal zone, representing a range of trade-offs between apparency and resistance. Those in the region of inadequate resistance should tend to become less apparent and/or more resistant. Those with "excess" resistance may tolerate higher levels of apparency or would be expected to reduce allocation to resistance. For any given level of apparency, the model suggests that the equilibrium level of resistance should reflect tissue value.

In most of the tree species (22 pioneers and 24 persistents) studied by Coley (1983), the young foliage was grazed at higher rates than the old foliage. The difference was correlated with changing toughness and content of water and nitrogen, as expected, though levels of tannins and total phenolics were higher in the young leaves. Also in accord with expectations was the finding that the mature foliage of pioneer saplings was grazed six times more rapidly than that of persistents and that persistents contained less nitrogen and water, were tougher and contained more phenolics, including tannins, than did pioneers. However, by examining patterns of damage distribution on natural trees and on saplings transplanted at various densities, Coley (1983) was unable to find any evidence that the saplings of persistents and pioneers differed in their apparency to insects.

Coley's results led her to formulate another hypothesis, based on habitat quality, to account for interspecific differences in plant resistance. According to this "resource availability hypothesis" (Coley et al., 1985), plants adapted to favorable habitats (such as light gaps) can grow fast enough to survive the relatively high levels of damage resulting from low levels of defense. Plants that typically occupy unfavorable habitats are limited in their productivity, cannot grow so fast and must rely on greater resistance to herbivores. Coley (1983) showed that pioneer saplings produced more leaves per unit time than did persistents and that the natural life span of these leaves was shorter. A given amount of damage would thus have less impact on a pioneer since it results in a smaller percent loss of the plant's net primary production. The overall leaf value of pioneers is thus lower, and the tissues should therefore be less well defended.

The resource availability hypothesis, elaborated further by Coley et al. (1985), proposes more generally that the availability of resources in the environment is the major determinant of both the amount and type of plant defense. When resources are limited, plants with inherently slow growth are favored and the slow growth in turn favors high levels of defense. The hypothesis is consistent with Janzen's (1974) interpretation of the high levels of defenses occurring in the foliage of tropical trees growing on nutrient-poor sites.

d) Reconciliation of hypotheses. There is widespread agreement that many patterns of variation in the life histories of organisms can be predicted rather well in terms of two primary environmental variables, namely adversity and disturbance (Grime, 1977; Southwood, 1977; Coley, 1987). Since the habitat-quality hypothesis for plant defense allocation refers primarily to the adversity (resource availability) axis, while the apparency hypothesis is relevant to the disturbance axis, Southwood et al. (1986) and Coley (1987) have suggested that the predictions of the two hypotheses can be integrated in terms of the habitat templet model (see also Southwood, 1988). Thus ephemeral herbaceous species, the "ruderals" of Grime (1977), are predicted to have low levels of resistance on the basis of both high resource availability and low apparency (Fig. 2). At the other extreme, plants occupying stable but adverse environments (Grime's "stress tolerators") maintain high levels of resistance by virtue of low availability of resources and high apparency. In the stable, undisturbed habitats occupied by Grime's "competitors", the high levels of defense predicted on the basis

of low disturbance are offset by the low predictions derived from high levels of resource availability (Fig. 2). It is in these productive and stable habitats that biological interactions among species are likely to be best developed (Greenslade, 1983; Southwood, 1988). These would certainly seem to be the habitats in which plants may gain protection most dependably from mutualistic associations with natural enemies. Habitats that are simultaneously highly adverse and disturbed were believed by Grime (1977) to be essentially uninhabitable by plants (Fig. 2).

The habitat templet model has considerable value as an aid to predicting general patterns of defense allocation in different environments. One problem with the model, however, is that apparency is not related simply to disturbance. In communities subject to high fire frequency, for example, plants of disturbed habitats may actually be more apparent to herbivores than are plants of late successional stages (P. Marks, personal communication). Another drawback of the model is its failure to take into account differences in apparency that occur within a habitat or that may

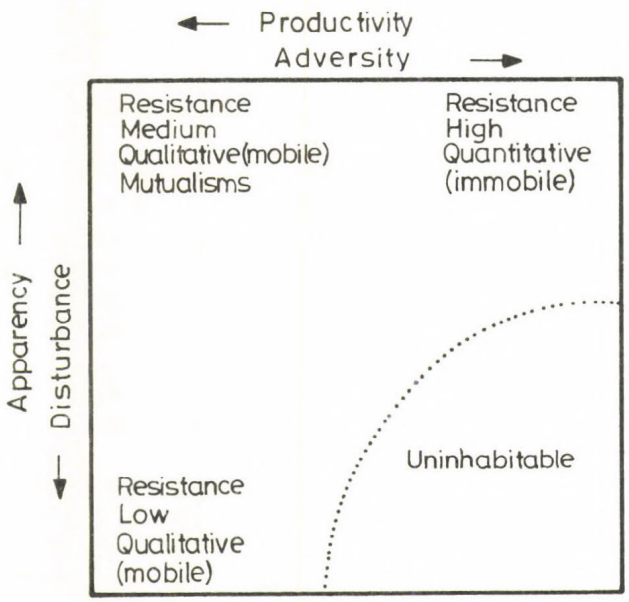

Fig. 2 Habitat templet model of plant resistance in relation to habitat adversity and disturbance (after Grime 1977, Greenslade 1983, Southwood et al. 1986, Southwood 1988, Coley 1987).

be otherwise unrelated to disturbance. Two species of *Aristolochia*, for example, occur together as herbaceous perennials in the longleaf pine forests of southeast Texas. Both are exposed to high levels of attack by their chief herbivore, the pipevine swallowtail butterfly, *Battus philenor* (Rausher, 1978; Rausher and Feeny, 1980). One species is able to survive because the toughness and low nutrient content of the mature leaves render them resistant to *Battus* larvae (Rausher, 1981a). The leaves of the other species, however, do not toughen and they remain vulnerable to *Battus* at all stages of growth. This species apparently survives in the habitat by virtue of its different, grass-like leaf shape that renders the plants less apparent to ovipositing *Battus* females (Rausher, 1978, 1981b). The different trade-offs between resistance and escape adopted by the two *Aristolochia* species are not readily explicable in terms of resource availability but are consistent with the apparency hypothesis.

It may be impossible to devise a model that incorporates all the factors that influence ecological patterns of plant defense. As yet, however, I remain unconvinced that such patterns cannot be explained on the basis of tissue value and apparency or vulnerability (*sensu* McKey, 1974).

Coley et al. (1985) have shown clearly that resource availability has a major influence on plant growth rate. The fast growth rates found in favorable habitats are associated with short leaf lifetimes and low leaf value, while the slow growth rates found in adverse habitats are associated with long leaf lifetimes and high leaf value (Coley, 1983, 1987). The observed defense levels (low in favorable habitats, high in unfavorable ones) would thus seem to be in accordance with an interspecific version of the tissue value hypothesis. They may also be in accord with predictions on the basis of apparency. Though the short-lived leaves of fast-growing plants often suffer higher rates of damage than do the leaves of slow-growing plants, the total lifetime damage of long-lived leaves may actually be greater (Southwood et al., 1986). Furthermore, since measurements of damage are uncertain estimates of apparency, we cannot be confident that the prolonged exposure of persistent leaves in unfavorable habitats would not result in catastrophic damage if the leaves were less well defended. To the extent that plants can escape from herbivores and pathogens by fast growth, resource availability, through its influence on growth rate, may alter the relative dependence of plants on resistance and escape.

ACKNOWLEDGEMENTS

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REFERENCES

Bentley, B. L. (1977): Extrafloral nectaries and protection by pugnacious bodyguards. *Ann. Rev. Ecol. Syst. 8,* 407-427.

Berenbaum, M. (1978): Toxicity of a furanocoumarin to armyworms: case of biosynthetic escape from insect herbivores. *Science 201,* 532-534.

Berenbaum, M. (1980): Adaptive significance of midgut pH in larval Lepidoptera. *Am. Nat. 115,* 138-146.

Berenbaum, M. (1981a): Effects of linear furanocoumarins on an adapted specialist insect (*Papilio polyxenes*). *Ecol. Entomol. 6,* 345-351.

Berenbaum, M. (1981b): Patterns of furanocoumarin production and insect herbivory in a population of wild parsnip (*Pastinaca sativa* L.). *Oecologia 49,* 236-244.

Berenbaum, M. (1983a): Coumarins and caterpillars: A case for coevolution. *Evolution 37,* 163-179.

Berenbaum, M. R. (1983b): Effects of tannins on growth and digestion in two species of papilionids. *Entomol. exp. appl. 34,* 245-250.

Bernays, E. A. (1978): Tannins: an alternative viewpoint. *Entomol. exp. appl. 24,* 44-53.

Bernays, E. A. (1981): Plant tannins and insect herbivores: an appraisal. *Ecol. Entomol. 6,* 353-360.

Bernays, E. A. (1987): The evolution of deterrent responses in plant-feeding insects. In: R. F. Chapman, E. A. Bernays and J. G. Stoffolano, Jr. (eds), *Perspectives in Chemoreception and Behavior.* Springer-Verlag, New York. 159-173.

Bernays, E. A.and D. J. Chamberlain (1980): A study of tolerance of ingested tannin in *Schistocerca gregaria. J. Insect Physiol. 26,* 415-420.

Bernays, E. A. and M. Graham (1988): On the evolution of host specificity in phytophagous arthropods. *Ecology 69,* 886-892.

Blau, P. A., P. Feeny, L. Contardo and D. Robson (1978): Allylglucosinolate and herbivorous caterpillars: A contrast in toxicity and tolerance. *Science 200,* 1296-1298.

Bryant, J. P., F. S. Chapin, III and D. R. Klein (1983): Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos 40,* 357-368.

Cates, R. G. and G. H. Orians (1975): Successional status and the palatability of plants to generalized herbivores. *Ecology 56,* 410-418.

Chapman, R. F. and E. A. Bernays (1977): The chemical resistance of plants to insect attack. *Pontificiae Academiae: Scientarum Scripta Varia 41,* 1-40.

Coley, P. D. (1983): Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecol. Monogr. 53,* 209-233.

Coley, P. D. (1987): Interspecific variation in plant anti-hebivore properties: The role of habitat quality and rate of disturbance. *New Phytol. 106,* (Suppl.), 251-263.

Coley, P. D., J. P. Bryant and T. Chapin (1985): Resource availability and plant antiherbivore defense. *Science 22,* 895-899.

Damman, H. (1987): Leaf quality and enemy avoidance by the larvae of a pyralid moth. *Ecology 68,* 88-97.

Ehrlich, P. R. and P. H. Raven (1964): Butterflies and plants: a study in coevolution. *Evolution 18,* 586-608.
Feeny, P. P. (1968): Effect of oak leaf tannins on larval growth of the winter moth *Operophtera brumata. J. Insect Physiol. 14,* 805-817.
Feeny, P. P. (1969): Inhibitory effect of oak leaf tannins on the hydrolysis of proteins by trypsin. *Phytochem. 8,* 2119-2126.
Feeny, P. (1970): Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology 51,* 565-581.
Feeny, P. (1975): Biochemical coevolution between plants and their insect herbivores. In: L. E. Gilbert and P. H. Raven (eds), *Coevolution of Animals and Plants,* University of Texas Press, Austin. 3-19.
Feeny, P. (1976): Plant apparency and chemical defense. *Rec. Adv. Phytochem. 10,* 1-40.
Feeny, P. (1977): Defensive ecology of the cruciferae. *Ann. Missouri Bot. Gard. 64,* 221-234.
Feeny, P. (1983): Coevolution of plants and insects. In: D. L. Whitehead and W. S. Bowers (eds), *Natural Products for Innovative Pest Management,* Pergamon Press, Oxford. 167-185.
Feeny, P. (1990): Chemical constraints on the evolution of swallowtail butterflies. In: P. W. Price, T. M. Lewinsohn, W. W. Benson and G. W. Fernandes (eds), *Herbivory: Tropical and Temperate Perspectives,* Wiley, New York. (in press).
Feeny, P. P. and H. Bostock (1968): Seasonal changes in the tannin content of oak leaves. *Phytochem. 7,* 871-880.
Fox, L. R. and B. J. Macauley (1977): Insect grazing on *Eucalyptus* in response to variation in leaf tannins and nitrogen. *Oecologia 29,* 145-162.
Fraenkel, G. S. (1959): The raison d'être of secondary plant substances. *Science 129,* 1466-1470.
Futuyma, D. J. (1976): Food plant specialization and environmental predictability in Lepidoptera. *Am. Nat. 110,* 285-292.
Futuyma, D. J. (1983): Evolutionary interaction among herbivorous insects and plants. In: D. J. Futuyma and M. Slatkin (eds), *Coevolution.* Sinauer Associates, Sunderland, Massachussetts. 207-231.
Goldstein, J. L. and T. Swain (1965): The inhibition of enzymes by tannins. *Phytochem. 4,* 185-192.
Greenslade, P. J. M. (1983): Adversity selection and the habitat templet. *Am. Nat. 122,* 352-365.
Grime, J. P. (1977): Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat. 111,* 1169-1194.
Hairston, N. G., F. E. Smith and L. B. Slobodkin (1960): Community structure, population control and competition. *Am. Nat. 94,* 421-425.
Hedin, P. A. (ed) (1983): *Plant Resistance to Insects.* ACS Symposium Series, No. 208, American Chemical Society, Washington, D. C.
Janzen, D. H. (1966): Coevolution of mutualism between ants and acacias in Central America. *Evolution 20,* 249-275.

Janzen, D. H. (1973): Community structure of secondary compounds in plants. *Pure Appl. Chem. 34,* 529-538.
Janzen, D. H. (1974): Tropical blackwaters rivers, animals, and mast fruiting by the Dipterocarpaceae. *Biotropica 6,* 69-103.
Jermy, T. (1984): Evolution of insect/host plant relationships. *Am. Nat. 124,* 609-630.
Jermy, T. (1988): Can predation lead to narrow food specialization in phytophagous insects? *Ecology 69,* 902-904.
Levin, D. A. (1971): Plant phenolics: An ecological perspective. *Am. Nat. 105,* 157-181.
Levin, D. A. (1976): The chemical defences of plants to pathogens and herbivores. *Ann. Rev. Ecol. Syst. 7,* 121-159.
Martin, M. M. and J. S. Martin (1984): Surfactants: Their role in preventing the precipitation of proteins by tannins in insect guts. *Oecologia 61,* 342-345.
Maxwell, F. G. and P. R. Jennings (eds) (1980): *Breeding Plants Resistant to Insects*, John Wiley and Sons, New York.
McKey, D. (1974): Adaptive patterns in alkaloid physiology. *Am. Nat. 108,* 305-320.
McKey, D. (1979): The distribution of secondary compounds within plants. In: G. A. Rosenthal and D. H. Janzen (eds), *Herbivores: Their Interaction with Secondary Plant Metabolites*, Academic Press, New York. 56-133.
McNaughton, S. J. and J. L. Tarrants (1983): Grass leaf silicification: Natural selection for an inducible defense against herbivores. *Proc. Natl. Acad. Sci. 80:* 790-791
Mooney, H. A., S. L. Gulmon and N. D. Johnson (1983): Physiological constraints on plant chemical defenses. In: P. A. Hedin (ed), *Plant Resistance to Insects*, ACS Symposium Series 208, American Chemical Society, Washington, D. C. 21-36.
Moran, N. and W. D. Hamilton (1980): Low nutritive quality as defense against herbivores. *J. Theor. Biol. 86,* 247-254.
Price, P. W., C. E. Bouton, P. Gross, B. A. McPheron, J. N. Thompson and A. E. Weis (1980): Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Ann. Rev. Ecol. Syst. 11,* 41-65.
Pridham, J. B. (ed.) (1963): *Enzyme Chemistry of Phenolic Compounds*. Pergamon, Oxford, and Macmillan, New York.
Raupp, M. J. and R. F. Denno (1983): Leaf age as a predictor of herbivore distribution and abundance. In: R. F. Denno and M. S. McClure (eds), *Variable Plants and Herbivores in Natural and Managed Systems*, Academic Press, New York, 91-124.
Rausher, M. D. (1978): Search image for leaf shape in a butterfly. *Science 200,* 1071-1073.
Rausher, M. D. (1981a): Host plant selection by *Battus philenor* butterflies: The roles of predation, nutrition, and plant chemistry. *Ecol. Monogr. 51,* 1-20.
Rausher, M. D. (1981b): The effect of native vegetation on the susceptibility of *Aristolochia reticulata* (Aristolochiaceae) to hebivore attack. *Ecology 62,* 1187-1195.

Rausher, M. D. and P. P. Feeny (1980): Herbivory, plant density and plant reproductive success: The effect of *Battus philenor* on *Aristolochia reticulata*. *Ecology 61,* 905-917.

Reese, J. C., B. G. Chan and A. C. Waiss, Jr. (1982): Effects of cotton condensed tannin, maysin (corn) and pinitol (soybeans) on *Heliothis zea* growth and development. *J. Chem. Ecol. 8,* 1429-1436.

Rehr, S. S., P. P. Feeny and D. H. Janzen (1973): Chemical defence in Central American non-ant-acacias. *J. Anim. Ecol. 42,* 405-416.

Rhoades, D. F. and R. G. Cates (1976): Toward a general theory of plant antiherbivore chemistry. *Rec. Adv. Phytochem. 10,* 168-213.

Rosenthal, G. A. and D. H. Janzen (eds) (1979): *Herbivores: Their Interaction with Secondary Plant Metabolites*. Academic Press, New York.

Scriber, J. M. (1977): Limiting effects of low leaf-water content on the nitrogen utilization, energy budget, and larval growth of *Hyalophora cecropia* (Lepidoptera: Saturniidae). *Oecologia 28,* 269-287.

Scriber, J. M. and P. Feeny (1979): Growth of herbivorous caterpillars in relation to feeding specialization and to the growth forms of their food plants. *Ecology 60,* 829-850.

Scriber, J. M. and F. Slansky, Jr. (1981): The nutritional ecology of immature insects. *Ann. Rev. Entomol. 26,* 183-211.

Seigler, D. and P. W. Price (1976): Secondary compounds in plants: Primary functions. *Am. Nat. 110,* 101-105.

Slansky, F. Jr. and P. Feeny (1977): Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food-plants. *Ecol. Monogr. 47,* 209-228.

Southwood, T. R. E. (1972): The insect/plant relationship: An evolutionary perspective. In: H. F. van Emden (ed), *Insect/Plant Relationships.* Blackwell Scientific Publications, Oxford. 3-30.

Southwood, T. R. E. (1977): Habitat, the templet for ecological strategies? *J. Anim. Ecol. 46,* 337-365.

Southwood, T. R. E. (1988): Tactics, strategies and templets. *Oikos 52,* 3-18.

Southwood, T. R. E., V. K. Brown and P. M. Reader (1986): Leaf palatability, life expectancy and herbivore damage. *Oecologia 70,* 544-548.

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INSECT HERBIVORE POPULATION DYNAMICS: IS A NEW PARADIGM AVAILABLE?

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ABSTRACT

A broader synthesis than is now available at the population dynamics level of plant-insect herbivore interactions and three-trophic-level systems will be achieved by recognizing continua of interactions. The continua include the range from latent to eruptive species of insect herbivores, the range from favorable hosts being vigorous to stressed, population dynamics on vegetation ranging from early to late succession, and the range in insect species that are lightly to heavily impacted by natural enemies. The extremes of these continua seem to be correlated mechanistically with latent species favored by vigorous plant growth in early succession, with little impact by natural enemies. Eruptive species seem to be favored by mature, old, or stressed plants in late succession, where natural enemies can become abundant.

The mechanistic explanation for these patterns invokes a flow of effects from evolved and primitive characteristics of species that limit plant resource exploitation, the Phylogenetic Constraints. In response to these, a species evolves a set of adaptations that minimizes constraints, the Adaptive Syndrome. In turn, these evolved characters largely dictate the ecology, including population dynamics, of the insect herbivore, which become Emergent Properties of the system. Such scenarios are presented for the latent shoot-galling sawfly, *Euura lasiolepis,* and contrasted with the eruptive Eastern spruce budworm, *Choristoneura fumiferana*. This new view of insect herbivore population dynamics provides a basis for broad comparative studies on species ranging from latent to eruptive. Emphasis is on female behavior and natality in response to plant resource quality. This view may prove to be an alternative paradigm to the commonly used life-table approach which has emphasized proximate ecological factors, especially mortality, without an evolutionary and behavioral perspective.

Key words: Adaptive syndrome, *Choristoneura fumiferana,* continua of interaction, emergent properties, eruptive species, *Euura lasiolepis,* female behavior, latent species, life tables, natality, natural enemies, phylogenetic constraints, plant resource quality, plant stress, plant vigor, shoot-galling sawfly, spruce budworm, three-trophic-level systems, vegetational succession.

INTRODUCTION

A general theory on plant-herbivore interactions must address the now evident continua of dynamical responses of insect herbivores to plant resources, both in time and space. These continua include the following:

1. The range in herbivore population dynamics from species with latent or none-ruptive popultions, to species which commonly erupt.

2. The range in herbivore population responses from positive on old or stressed plants to negative, and in some associations the strong positive association between vigorous plant growth and herbivore attack and survival.

3. The range in patterns of population dynamics over the landscape, from early successional patches to extensive mature forest.

4. An additional continuum involves responses of natural enemies to these continua, ranging from being unimportant in population dynamics to playing a significant role in regulation.

Can one general conceptual framework encompass all this variation? Here I make the beginnings of an attempt to do so. Ultimately, I believe a successful synthesis could act as a major focus for future development of this field.

Progress in examining aspects of the for continua above has been mixed. The study of differences between latent and eruptive species has become more focused in recent years, with real progress made in how comparative approaches can yield important insights (e.g. Rhoades, 1985; Hanski and Otronen, 1985; Hanski, 1987; Mason, 1987; Nothnagle and Schultz, 1987; Wallner, 1987; Myers, 1988; Price et al., 1990). However, there is no synthesis yet on the fundamental differences between latent and eruptive species, although much interest in the subject exists. A major limiting factor has been the shortage of detailed studies on latent species.

The second continuum needing integration into a general theory is the role of plant vigor and stress, and the range of responses by insect herbivores. Even recently Mattson and Haack (1987a) had to state that the evidence for a link between plant water stress and insect outbreaks was largely circumstantial. However, we know that there is some kind of link between conditions that include plant stress and insect outbreaks, and the literature is well reviewed (e.g. Mattson and Haack, 1987a, b; Heinrichs, 1988). The mechanistic approach advocating six lines of influence from stressed plants to insect behavior and physiology (Mattson and Haack, 1987b) should provide the focus needed for understanding the role of stress. Much less attention has been paid to insect herbivores that are favored by vigorous plants or vigorous plant parts, and I hope our review (Price et al., 1990) will generate wide recognition that many herbivore species respond negatively to plant stress. White (1978, p. 77) stated that "Most sorts of herbivores have become adapted to feeding only on the highly nutritious 'flush' tissue produced by plants during periods of fast growth." Therefore, we need to understand better the patterns of herbivore use on the range of resources supplied by plants.

A landscape view of vegetational pattern and insect herbivore population dynamics should also play a role in the development of general theory. The theory of plant apparency generated by Feeny (1975, 1976) and Rhoades and Cates (1976) provided a major stimulus to research on plant-herbivore interactions partly because it adopted a landscape perspective for understanding pattern. The alternative hypothesis on resource availability to plants as the major determinant of pattern in plant defenses also emphasizes landscape (Coley, 1983; Coley et al., 1985). But the theory has not developed to the point where superimposed on these patterns is an under-

standing of how insect herbivores respond to these patterns at the level of their population dynamics.

The role of natural enemies in insect herbivore population dynamics, the fourth continuum, is very hard to generalize about, and therefore difficult to integrate into general approaches. The main problem is a prevalence of correlational studies lacking experimental tests of mechanisms (Price, 1987). Nevertheless, we do know of cases in which insect herbivores are kept very rare by natural enemies, and the cases in which enemies play almost no role in the host population dynamics. A continuum of effects from very strong to very weak exists. Strong effects by parasitoids exist in Europe on the mountain-ash sawfly, *Pristiphora geniculata* (Eichhorn and Pschorn-Walcher, 1978). It escaped its natural enemies in Canada, became epidemic, but when the association was reestablished, regulation at low densities prevailed (Quednau, 1984). Other such cases exist. Moderate impact probably prevails in bark beetle systems in which natural enemies may influence the height of peak populations, but not the driving forces and general patterns of eruptions (Berryman, 1982). Very weak effects of natural enemies are seen in the shoot-galling sawfly, *Euura lasiolepis,* in which galls become too large for small pteromalid parasitoids to attack effectively (Price and Clancy, 1986; Price, 1988) or too hard to penetrate for a larger ichneumonid (Craig et al., 1989b). More research should be devoted to identifying pattern in the range of responses of natural enemies to insect herbivore populations.

Integration of these four continua may be advanced by a new perspective we have proposed (Price et al., 1990), which is developed in the next section.

PHYLOGENETIC CONSTRAINTS, ADAPTIVE SYNDROMES, AND EMERGENT PROPERTIES

We have argued that there is a flow of influence from primitive evolved characters that are in some way limiting to exploitation of plants, to adaptive responses that minimize the limiting characters, to the ecological performance of the species at the behavioral and physiological level, and ultimately to the population dynamics of the species. The limiting primitive characters we call the *Phylogenetic Constraints*. The adaptive responses to these constraints we call the *Adaptive Syndrome*, and these evolved characters influence profoundly many other characters, including the population dynamics, which we call *Emergent Properties* (Price et al., 1990). This is an unconventional view because it argues that much of the population dynamics of an insect herbivore species is dictated by ancient phylogenetic constraints, and the proximate ecological factors that researchers have concentrated on over the past 50-60 years or so are largely results of evolved characters, not causes of the dynamics. This is such a departure from conventional views that I would like to think of it as an alternative paradigm that must now compete for support with the conventional, strongly ecological and proximate view that has prevailed since population dynamics studies were initiated about 100 years ago (e.g. Howard, 1987). The conventional view was embodied in the development of life tables which summarized well mortality

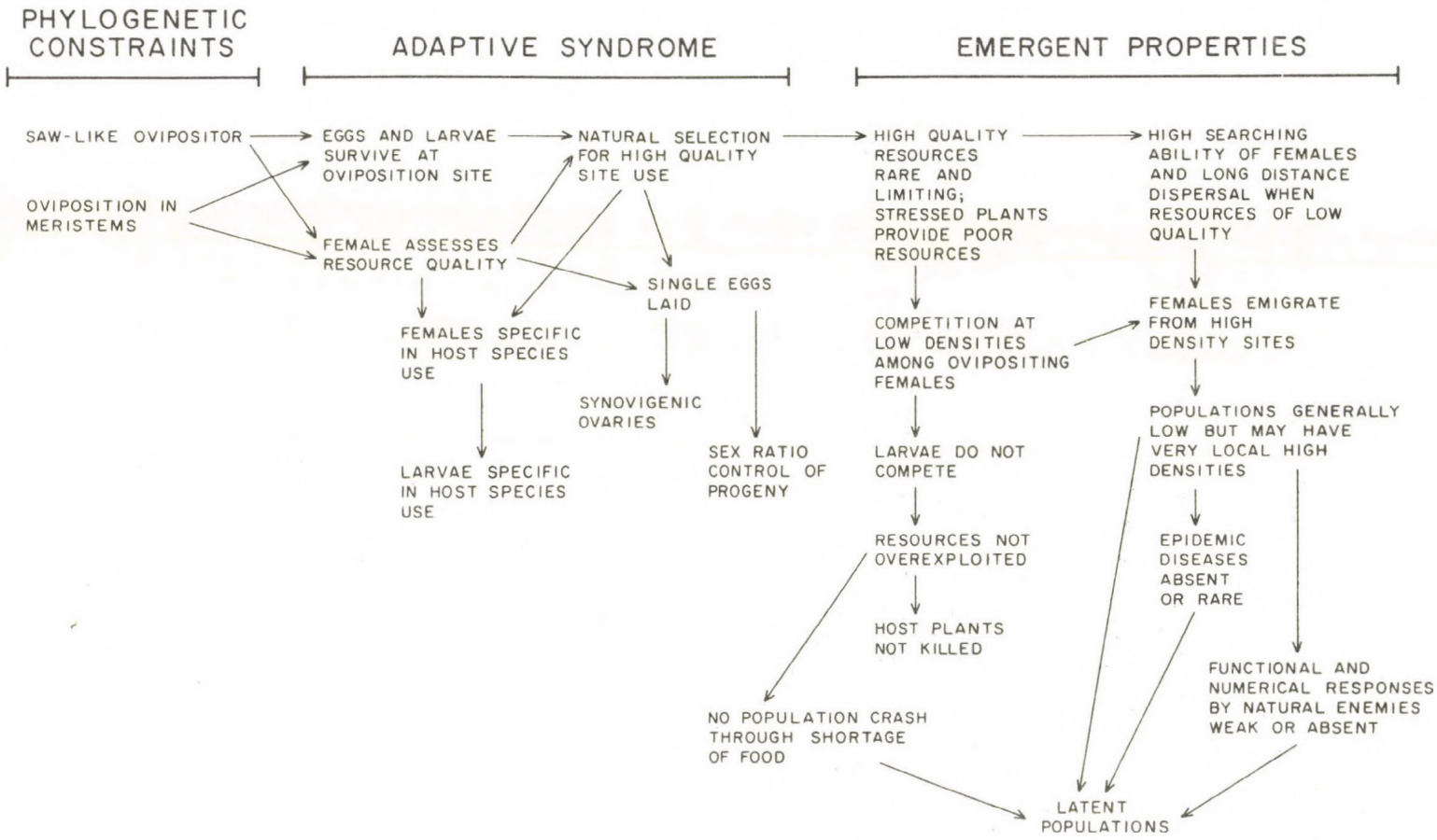

Fig. 1 Relationships among phylogenetic constraints, adaptive syndrome, and emergent properties, resulting in typically latent populations of *Euura lasiolepis* (modified from Price et al., 1990)

factors impinging on a cohort of insects, but were less effective in capturing the role of female behavior and natality, especially in response to plant resource variation (Price et al., 1990).

Two examples will serve to illustrate the flow of influence from phylogenetic constraints to adaptive syndrome, to emergent properties. The first is on the shoot galling sawfly, *Euura lasiolepis*. It is a latent species with populations varying over less than two orders of magnitude in any one site (Price et al., 1990). The second example is on the Eastern Spruce Budworm, *Choristoneura fumiferana*, an eruptive species, varying in population size between three to five orders of magnitude when moving from endemic to epidemic levels.

The saw-like ovipositor of sawflies is an ancient characteristic found in the first fossil sawflies in the Triassic period, 225 million years ago. This is a phylogenetic constraint because it limits oviposition to internal parts of plants which must be soft and easily penetrated (Fig. 1). In addition, for galling species like *Euura lasiolepis* rapidly developing meristems are conducive to gall development (Weis et al., 1988). Hence, females attack rapidly growing shoots and are very selective in oviposition (Craig et al., 1986). Eggs and larvae must survive at the oviposition sites, reinforcing female selectivity for shoots resulting in high survival among progeny (Preszler and Price, 1988; Craig et al., 1989a). This linkage between survival of progeny at the site of oviposition, the female's ability to evaluate resource quality, and therefore, natural selection for high quality site use, becomes the core of the adaptive syndrome of the species (Fig. 1). Additional evolved traits in the syndrome include high specificity in host plant species use by both females and larvae, and the oviposition of single eggs between bouts of searching for rare long shoots, so that synovigenic ovaries are adaptive. Eggs are produced gradually during the life of the insect (Flanders, 1950; Jervis and Kidd, 1986). *Euura* is an arrhenotokous species, making sex ratio control by the female possible in response to plant quality, when coupled with oviposition of single eggs, and resource evaluation by the female.

The emergent properties then follow almost inevitably from the evolved basis of the plant-hebivore interaction. High quaility resources are rare and limiting (Fig. 1), and females compete at low densities for high quality sites (Craig et al., 1989c). As a result larvae do not compete, resources are not overexploited, and herbivore impact on plant fitness is generally low (Sacchi et al., 1988). A population can persist for many years on a willow clone if rapidly-growing-shoot production is sustained. But the population has a clearly defined carrying capacity, based on the density of long shoots, with strong and rapid negative feedback on population growth imposed by competition for oviposition sites. The population is constrained, with latent populations prevailing almost indefinitely (Fig. 1).

Natural enemies have a weak effect on populations of *Euura*. Because populations are so stable, functional and numerical responses by enemies are absent or difficult to detect. On high quality shoots, galls become large and limit access to small parasitoids (Price and Clancy, 1986; Price, 1988). Galls also toughen too rapidly to allow extensive access by larger parasitoids, limiting their efficacy (Craig et al., 1989b). In caging experiments over three generations, *Euura* populations did not

Table 1 Examples of insect herbivores that probably exhibit latent population dynamics (sources are in Price et al., 1990).

Herbivore species	Host plant genus	Feeding location	Plant resources tatus	Female adult selects larval feeding site
1. *Euura exiguae*	*Salix*	in shoot gall	young, vigorous	yes
2. *Euura mucronata*	*Salix*	in bud galll	young, vigorous	yes
3. *Pemphigus betae*	*Populus*	in leaf gall	large leaves	yes
4. *Diplolepis spinosa*	*Rosa*	in shoot gall	young, vigorous	yes
5. *Rhynchites betulae*	*Betula*	in leaf roll	young plants	yes
6. *Dioryctria ponderosae*	*Pinus*	in shoot	young plants, large shoots	yes
7. *Rhyacionia frustrana*	*Pinus*	in shoot	young plants	yes
8. *Eucosma gloriola*	*Pinus*	in shoot	young plants	yes
9. *Pissodes strobi*	*Pinus*	in shoot	young plants	yes
10. *Pissodes terminalis*	*Pinus*	in shoot	young plants	yes

differ significantly among treatments of uncaged, caged without parasitoids, and caged with parasitoids (Woodman and Price, 1990). Therefore, the scenario for this latent species relating to natural enemies is justified (Fig. 1).

In fact, *Euura lasiolepis* is probably one of the best studied latent species available for developing a continuum approach to insect herbivore population dynamics. Typically, latent species have been ignored because they are not economically important, and are frequently difficult to study because of low population levels (Mason, 1987). But it does seem to be representative of many herbivores that attack vigorous plant growth, many of which are endophytic, where females oviposit at the site in which eggs and larvae must survive (Price et al., 1990) (Table 1).

A contrasting scenario exists for the Eastern Spruce Budworm, *Choristoneura fumiferana*, which is eruptive (Fig. 2). The phylogenetic constraint, basic to the genus *Choristoneura*, is that females oviposit on well developed foliage which is not the foliage larvae will eventually feed upon. In addition, larvae commonly disperse on silken threads, and begin feeding in the following year on buds, male flowers, and newly flushing leaves (Morris, 1963; Mattson et al., 1988). Females, therefore, cannot evaulate foliage quality for larvae. Under such conditions larvae evolve with the capacity to survive on a wide variety of foliage conditions within a plant population and species, and a generalized feeding capability develops (Fig. 2).

The emergent properties for such an eruptive species are then extensive (Fig. 2). For a generalized herbivore, foliage is abundant and carrying capacities are consequently high, especially in mature forests. The system is permissive of high populations, in contrast to the constraining system for the latent species *Euura lasiolepis*. Sooner or later the full range of permitted population densities will be observed, and eruptions will occur. This scenario explains how eruptions can occur, not why they occur. An eruption can result in food shortage for larvae, death of trees, and an insect herbivore population crash. With large fluctuations in density, epidemic

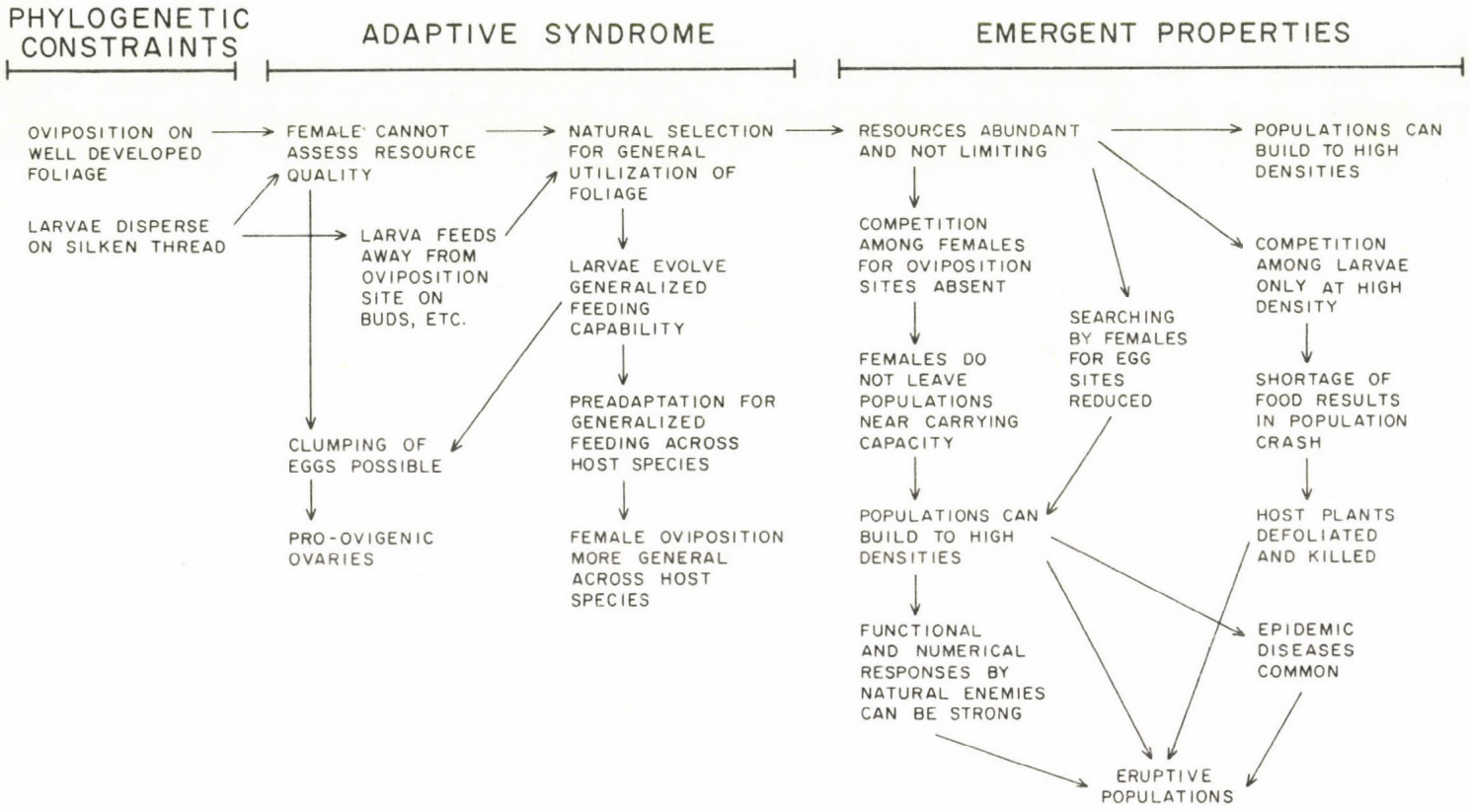

Fig. 2 Relationship among phylogenetic constraints, adaptive syndrome, and emergent properties, resulting in eruptive populations of *Choristoneura fumiferana* (modified from Price et al., 1990), typical of many eruptive species.

Table 2 Examples of insect herbivores that exhibit eruptive population dynamics (sources in Price et al., 1990)

Herbivore species	Host plant genus	Feeding location	Oviposition site selection and larval feeding linkage
Operophtera brumata	*Quercus*	On leaves	Poor. Females wingless, lay eggs in winter when foliage is absent.
Porthesia dispar	*Quercus* etc.	On leaves	Poor. Oviposition on bark, females do not fly, larvae disperse passively.
Malacosoma disstria	*Populus*	On leaves	Poor. Oviposition on bark, larva feeds following spring.
Alsophila pometaria	*Quercus* etc.	On leaves	Poor. Females wingless, oviposition in winter, passive dispersal of larvae.
Neodiprion swainei	*Pinus*	On leaves	Poor. Female poor flyer, proovigenic with heavy load of eggs, oviposition on young needles, larvae feed on old needles.
Orgyia pseudotsugata	*Pseudotsuga*	On leaves	Poor. Clusters of 150 eggs laid on female's cocoon, larvae disperse passively.
Phryganidia californica	*Quercus*	On leaves	Poor. Female weak flyer, 60 eggs per mass not neccessarily on leaves larvae will feed on.
Coloradia pandora	*Pinus*	On leaves	Poor. Eggs in clusters on needles or bark. Most feeding in second year of life cycle.
Paleacrita vernata	*Ulmus* etc.	On leaves	Poor. Females wingless. Eggs under bark, before foliage is available.
Lambdina punctata	*Quercus*	On buds & leaves	Poor. Eggs laid in leaf litter, and under bark scales.

diseases and other natural enemies are likely to show positive correlations with density, although cause and effect are hard to disentangle (Ewald, 1987; Price, 1987).

These contrasted scenarios for latent and eruptive species do not explain all aspects of the continuum from latent to eruptive in population dynamics. For example, all species of *Choristoneura* are not eruptive, and all populations are not eruptive within species with eruptive populations. Refinement of these broad scenarios is needed. However, many eruptive species share with the budworm the dislocation between female oviposition and larval feeding site (Table 2). So the scenario for spruce budworm captures many elements of the eruptive population type.

The ability to use this approach of phylogenetic constraints, adaptive syndromes, and emergent properties, for synthesizing the four continua of interaction discussed in the introduction, can be developed now in the next section.

CORRELATIONS AMONG CONTINUA IN PLANT-HERBIVORE INTERACTION

The sawfly and budworm examples probably represent the two extremes in population dynamics from very latent to very eruptive. They also illustrate ends of the continuum from herbivores that attack very vigorous plants and plant parts, to those

that show little or no discrimination of plant quality, and may respond positively to old and/or stressed plants. For species attacking woody plants, they also represent herbivores on early successional and late successional stages of vegetation. Regarding natural enemies, the sawfly illustrates one extreme of little or no enemy impact on population dynamics, and the budworm at least illustrates the correlation between high larval densities and high densities of parasitoids and predators (Morris, 1963; Clark et al., 1979; Holling, 1988).

Thus, at these rather extreme positions on the four continua, the characteristics within a species appear to be strongly correlated, making it simpler to synthesize what may seem to have been rather disparate aspects of insect herbivore systems. The generality of these patterns appears to be supported by the examples of latent species in Table 1, and eruptive species in Table 2, and additional examples in Price et al. (1990).

A realistic view of the primaeval terrestrial temperate landscape must include broad expanses of mature forest, interspersed with patches of recent disturbance in which young and vigorous regeneration occurs. Disturbance results from tree falls, fire, flooding, land slides, epidemic disease and herbivory, and the like. The latent population species type seems to be associated with disturbance vegetation in which growth is juvenile and vigorous, and the eruptive population species type apparently predominates in mature forest, if we restrict our perspective to natural woody-plant systems, and exclude agricultural systems. Eruptive species on woody plants generally occur in broad stands of mature forest, wherein, for species adapted as generalists on plant quality, very high carrying capacities exist.

The challenge that I see now is to test the extent to which the middle of these continua can be filled in, and the species of insect herbivore that belong between the extremes of the very latent and very eruptive. Real synthesis will be achieved only when the full range of the four continua are complete, and many species placed on these continua. Other factors will need to be considered also. For example, all relatives of eruptive species do not reach epidemic densities. Why? I have not been able to address this question yet. Are there general approaches to investigating differences between eruptive and latent species that are closely related, or must we study idiosynchrasies of the species and depart from a general conceptual framework and theory? Many questions need resolution.

However, I think there are some important points that are inherent in the scenarios for latent and eruptive species and the continua they exist on. They illustrate contrasting patterns in the population dynamics of the species. Many of these may need more study before species can be placed on the continua of interaction. The first is the role of plant resource quality and its distribution in insect herbivore population dynamics. For *Euura lasiolepis*, and probably for many other gallers and shoot borers, this seems to be of paramount importance in setting an apparently low carrying capacity for the herbivore population. Thus the dynamical properties of these kinds of herbivores are tightly regulated by plant resource quality. This may not be so for eruptive species where quantity of forest growth and maturity, providing

large standing crops of food, are probably more important than foliage quality (e.g. Clark et al., 1979).

The second point is the importance of female behavior and its influence on natality in the population. Again, in the latent species we have studied, female behavior in response to plant resource quality is a major key to understanding population dynamics (Preszler and Price, 1988; Craig et al., 1989a). Life table construction requires modification when the plant-herbivore interaction influences natality so profoundly (Preszler and Price, 1988; Price et al., 1990). Female oviposition behavior of eruptive species may be much less critical because decisions appear to be less important. The first life tables for the spruce budworm (Morris and Miller, 1954) may well have captured the essential information on natality, even though ignoring female behavior, because females are so unselective relative to those of latent species. But female behavior and natality do in general need more study in relation to insect herbivore population dynamics.

The third contrast is in the role of intraspecific competition in population dynamics. Females of latent species probably compete for oviposition sites, and if these are rare, competition will be important even at low densities (e.g. Craig et al., 1989c). As a result, larvae do not compete. Females of eruptive species probably do not compete for oviposition sites because they are relatively undiscriminating in site choice. As a result, larvae are likely to become dense, and shortage of food causes competition. An interesting point is that if we study communities of latent species and concentrate on larval interactions, interspecific competition may not be apparent, even though spacing of larvae by females has played an important role. This view needs more study in the context of the apparent weakness of competition in insect herbivore communities (Lawton and Strong, 1981; Strong et al., 1984). Strong et al. (1984) noted the infrequency of intraspecific competition as an important factor in phytophagous insect life table analysis, but a new concentration on female competition for high quality oviposition sites is needed in my opinion.

The fourth contrast between latent and eruptive species is in the way they relate to vegetational dynamics. They may well mimic the reproductive strategies of the plants themselves. The minute seeds of willow disperse widely and colonize newly disturbed ground. Young vigorously growing plants in new sites are ideal for shoot-galling sawflies, so long distance dispersal by sawflies may well be an integral part of the adaptive syndrome of the species, even though a small proportion of a population may be involved. Given enough stability, mature forest tends to replace itself by seeds with limited dispersal. Insect populations can persist in such stable settings with a high carrying capacity, with reduced needs for dispersal, and flightlessness becomes a common feature of forest insects (e.g. Table 2).

Patterns in herbivore use of vegetation also extend the concepts of plant apparency (Feeny, 1976; Rhoades and Cates, 1976) and resource availability in plant defense evolution (Coley, 1983; Coley et al., 1985). Rapidly growing plant species frequently grow in early successional habitats, have relatively low levels of chemical defense, but can readily compensate for damage by regrowth in high resource environments. I argue that on these plants latent species of insect herbivores will predominate. In

low resource conditions, plant species with slow growth will prevail, with high levels of chemical defenses, and these will occur commonly in late stages of vegetational succession (Coley et al., 1985). This is where the eruptive insect herbivores on woody plants tend to be concentrated.

This concept of the continua of interactions among plants and their insect herbivores needs much filling in and, no doubt, refinement to include the wide variety of insect herbivores, plant-herbivore interactions, and three-trophic-level systems seen in nature. More comparative experimental approaches to insect population dynamics will certainly be needed to test the reality of relevant adaptive syndromes and emergent properties. Current studies in my research group on gallers, leaf miners and external leaf feeders are generally supportive of the mechanistic links between phylogenic constraints, adaptive syndromes and emergent properties. These views do offer an alternative to the current paradigm of insect herbivore population dynamics which has emphasized mortality imposed on cohorts in ecological time in the absence of an ultimate view of why such susceptibility to mortality should occur. Whether a more evolutionary view, as proposed here, will compete successfully for paradigmatic status in the development of insect herbivore population dynamics remains to be seen.

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REFERENCES

Berryman, A. A. (1982): Population dynamics of bark beetles. In: J. B. Mitton and K. B. Sturgeon (eds), *Bark Beetles in North American Conifers*. University of Texas Press, Austin. 264-314.

Clark, W. C., D. D. Jones and C. S. Holling (1979): Lessons for ecological policy design: A case study of ecosystem management. *Ecol. Modelling 7,* 1-53.

Coley, P. D. (1983): Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecol. Monogr. 53,* 209-233.

Coley, P. D., J. P. Bryant and F. S. Chapin (1985): Resource availability and plant antiherbivore defense. *Science 230,* 895-899.

Craig, T. P., P. W. Price and J. K. Itami (1986): Resource regulation by a stem-galling sawfly on the arroyo willow. *Ecology 67,* 419-425.

Craig, T. P., J. K. Itami and P. W. Price (1989a): A strong relationship between preference and performance in a shoot-galling sawfly to attack by a parasitoid. *Ecology* (in press)

Craig, T. P., J. K. Itami and P. W. Price (1989b): The window of vulnerability of a shoot-galling sawfly to attack by a parasitoid. *Ecology* (in press).

Craig, T. P., J. K. Itami and P. W. Price (1989c): Intraspecific competition and facilitation by a shoot-galling sawfly. *J. Anim. Ecol.* (in press)

Eichhorn, O. and H. Pschorn-Walcher (1978): Biologie und Parasiten der Ebereschen-Blattwespe, *Pristiphora geniculata* Htg. (Hym.: Tenthredinidae). *Z. ang. Entomol. 85,* 154-167.

Ewald, P. W. (1987): Pathogen-induced cycling of outbreak insect populations. In: P. Barbosa and J. C. Schultz (eds), *Insect outbreaks*. Academic, New York. 269-286.

Feeny, P. (1975): Biochemical coevolution between plants and their insect herbivores. In: L. E. Gilbert and P. H. Raven (eds), *Coevolution of Animals and Plants*. University of Texas Press, Austin. 3-19.

Feeny, P. (1976): Plant apparency and chemical defense. In: J. W. Wallace and R. L. Mansell (eds), *Biochemical Interaction between Plants and Insects*. Plenum, New York. 1-40.

Flanders, S. E. (1950): Regulation of ovulation and egg disposal in the parasitic Hymenoptera. *Can. Entomol. 82,* 134-140.

Hanski, I. (1987): Pine sawfly population dynamics: Patterns, processes, problems. *Oikos 50,* 327-335.

Hanski, I. and M. Otronen (1985): Food quality induced variance in larval performance: Comparison between rare and common pine-feeding sawflies (Diprionidae). *Oikos 44,* 165-174.

Heinrichs, E. A. (ed.) (1988): *Plant Stress-Insect Interactions*. Wiley, New York.

Holling, C. S. (1988): Temperate forest insect outbreaks, tropical deforestation and migrating birds. *Mem. Entomol. Soc. Can. 146,* 21-32.

Howard, L. O. (1897): A study of insect parasitism: a consideration of the parasites of the white-marked tussock moth, with an account of their habits and interrelations and with descriptions of new species. *U. S. Dept. Agr. Bureau Entomol. Tech. Series 5,* 1-57.

Jervis, M. A. and N. A. C. Kidd (1986): Host-feeding strategies in hymenopteran parasitoids. *Biol. Rev. 61,* 395-434.

Lawton, J. H. and D. R. Strong (1981): Community patterns and competition in folivorous insects. *Am. Nat. 118,* 317-338.

Mason, R. R. (1987): Nonoutbreak species of forest Lepidoptera. In: P. Barbosa and J. Schultz (eds), *Insect Outbreaks*. Academic, New York. 31-57.

Mattson, W. J. and R. A. Haack (1987a): The role of drought stress in provoking outbreaks of phytophagous insects. In: P. Barbosa and J. C. Schultz (eds), *Insect outbreaks*. Academic, New York. 365-407.

Mattson, W. J. and R. A. Haack (1987b): The role of drought in outbreaks of plant-eating insects. *BioScience 37,* 110-118.

Mattson, W. J., G. A. Simmons and J. A. Witter (1988): The spruce budworm in Eastern North America. In: A. A. Berryman (ed), *Dynamics of Forest Insect Populations*. Plenum, New York. 309-330.

Morris, R. F. (ed) (1963): The dynamics of epidemic spruce budworm populations. *Mem. Entomol. Soc. Can. 31,* 1-332.

Morris, R. F. and C. A. Miller (1954): The development of life tables for the spruce budworm. *Can. J. Zool. 32,* 283-301.

Myers, J. H. (1988): Can a general hypothesis explain population cycles of forest Lepidoptera? *Adv. Ecol. Res. 18,* 179-242.

Nothnagle, P. J. and J. C. Schultz (1987): What is a forest pest? In: Barbosa P. and J. C. Schultz (eds), *Insect Outbreaks*. Academic, New York. 59-80.

Preszler, R. W. and P. W. Price (1988): Host quality and sawfly populations: A new approach to life table analysis. *Ecology 69,* 2912-2020.

Price, P. W. (1987): The role of natural enemies in insect populations. In: P. Barbosa and J. C. Schultz (eds), *Insect Outbreaks*. Academic, New York. 287-312.

Price, P. W. (1988): Inversely density-dependent parasitism: The role of plant refuges for hosts. *J. Anim. Ecol. 57,* 89-96.

Price, P. W. and K. M. Clancy (1986): Interactions among three trophic levels: Gall size and parasitoid attack. *Ecology 67,* 1593-1600.

Price, P. W., N. Cobb, T. P. Craig, G. W. Fernandes, J. K. Itami, S. Mopper and R. W. Preszler (1990): Insect herbivore population dynamics on trees and shrubs: New approaches relevant to latent and eruptive species and life table development. In: E. A. Bernays (ed), *Insect-Plant Interactions*. Vol. 2. CRC Press, Boca Raton. (in press)

Quednau, F. W. (1984): *Pristiphora geniculata* (Htg.), Mountain-Ash Sawfly (Hymenoptera: Tenthredinidae). In: J. S. Kelleher and M. A. Hulme (eds), *Biological Control Programmes Against Insects and Weeds in Canada, 1969-1980.* Commonwealth Agricultural Bureaux, Farnham. 381-385.

Rhoades, D. F. (1985): Offensive-defensive interactions between herbivores and plants: Their relevance in herbivore population dynamics and ecological theory. *Am. Nat. 125,* 205-238.

Rhoades, D. F. and R. G. Cates (1976): Toward a general theory of plant antiherbivore chemistry In: J. W. Wallace and R. L. Mansell (eds), *Biochemical Interaction Between Plants and Insects.* Plenum, New York. 168-213.

Sacchi, C. F., P. W. Price, T. P. Craig and J. K. Itami (1988): Impact of shoot galler attack on sexual reproduction in the arroyo willow. *Ecology 69,* 2021-2030.

Strong, D. R., J. H. Lawton and R. Southwood (1984): *Insects on Plants: Community Patterns and Mechanisms*. Harvard University Press, Cambridge.

Wallner, W. E. (1987): Factors affecting insect population dynamics: Differences between outbreak and non-outbreak species. *Ann. Rev. Entomol. 32,* 317-340.

Weis, A. E., R. Walton and C. L. Crego (1988): Reactive plant tissue sites and the population biology of gall makers. *Ann. Rev. Entomol. 33,* 467-486.

White, T. C. R. (1978): The importance of relative shortage of food in animal ecology. *Oecologia 33,* 71-86.
Woodman, R. L. and P. W. Price (1990): Which factors influence insect herbivore community structure? : A test of enemy impact versus the abiotic environment. (In preparation)

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RAPID WOUND-INDUCED CHANGES IN PLANT CHEMISTRY: THEIR ECOLOGICAL SIGNIFICANCE TO INSECTS

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ABSTRACT

Many ecologist studying herbivory have regarded the plant as a virtually unlimited food resource for insects with the implication that intra-specific competition for this resource is rare and that predation and parasitism are the major regulatory factors. However, in this paper, evidence for wound-induced changes in plants following insect feeding is briefly reviewed, including discussion of the reasons why insects avoid damage. It is concluded that wound-induced effects lead to large numbers of small meals by invertebrate herbivores; these higher movement rates and more dispersed feeding involve the relative avoidance of young leaves and therefore have implications for the plant's fitness and the insects' survival.

Key words: wound-induced changes, insect movement, foraging, ecology.

INTRODUCTION

In 1983, Edwards and Wratten drew attention to the fact that at the end of the growing season in temperate regions the leaves of many woody and herbaceous plants typically bear large numbers of small holes, with defoliation of complete leaves being rare. However, when leaves are collected at the end of the season and analyses of these patterns are attempted, there is no obvious statistical null hypothesis with which to compare the apparent over-dispersion of damage. Since 1983, however, field and laboratory evidence involving insect behaviour has accumulated which suggests that most of these holes may be the result of the decision-making processes by foraging insects. In other words, the animals 'decide' where and for how long they will feed. Many ecologists studying herbivory have regarded the plant as a virtually unlimited food resource for insects, with the implication that intra-specific competition for this resource is rare and that predation and parasitism are the major regulatory factors (Hairston et al., 1960; Strong et al., 1984; Fowler and Lawton, 1985). The present paper will address three questions concerning insect feeding behaviour which are relevant to this ecological debate:

1. If it is true that foliage is present in such abundance why do the observed feeding patterns occur? i.e. what factors lead insects to abandon leaves after small amounts of feeding?

2. What is the behavioural basis of these patterns?

3. What are the consequences of such behaviour for the plant and for the herbivore?

The fact that the insects seem to take large numbers of small meals rather than a small number of large ones could be at least partially explained by any one of several processes, some of which are documented:

— evolved behaviour to avoid visually-hunting vertebrate predators by moving away from small amounts of damage (Heinrich, 1979);

— evolved behaviour to avoid detection by predators or parasitoids which respond to the plant chemicals associated with damage, leading again to the herbivores' moving away from such damage;

— background heterogeneity within and between leaves leading to foraging insects' continually abandoning low quality sites;

— diurnal rhythms in the herbivores' behaviour as an evolved adaptation to avoid foraging predators or parasitoids.

— interrupted meals brought about by successful or unsucessful predator or parasitoid attacks.

In this paper we are concerned principally with heterogeneity in food quality which is induced by the process of feeding; we examine the evidence that induced responses can cause insects to take large numbers of small meals, with higher levels of insect movement as a consequence, which can have implications both for plant fitness and herbivore survival.

WOUND-INDUCED CHANGES: SMALLER MEALS AND HIGHER MOVEMENT RATES

Evidence of wound-induced changes in leaves has grown in recent years and has been demonstrated in a wide range of plant species. The rate and scale of these changes can range widely from those confined within the leaf to those affecting the whole plant (Edwards and Wratten, 1983; Bergelson and Lawton, 1988). There is clear evidence that even small amounts of grazing may have rapidly developing consequences for the quality of the plant tissue in the vicinity of damage. Initially, results of this type were derived from visual assessments of insect damage at a range of distances from experimentally-imposed holes in leaves (e.g. Gibberd et al., 1988). More recently, Croxford et al. (1989) used computer analysis of composite video images to demonstrate the way in which Lepidoptera larvae avoided damaged parts of leaves of cotton and soybean. In the field, Silkstone (1987) demonstrated that leaves that had received small amounts of artificial damage in the spring received less grazing by natural herbivores in the subsequent months. Also, a higher proportion of these previously damaged leaves escaped all subsequent natural grazing than did the controls. These laboratory and field results could help to explain the often apparently highly dispersed patterns of grazing within and between leaves. A problem of interpretation of these results is that the avoidance of damaged areas by insects

may be a consequence of one or more of the processes listed in the introduction above. However, several lines of evidence eliminate the possibility that insects are merely avoiding physical damage and point towards the animals' avoiding chemically induced leaves or parts of leaves:

1. responses differ between plant species in the same community (Edwards et al., 1986);

2. there is a clear seasonal effect in that tree leaves damaged in the summer or later show a much reduced effect compared with leaves damaged in the spring (Wratten et al., 1984);

3. the age of the leaf influences the strength of the wound-induced change; young leaves appear to show the effect much more strongly than do old leaves on the same shoot (Edwards et al., 1990);

4. the effect of the damage on foraging larvae declines with time following damage (Gibberd et al., 1988);

5. the effect is often transmitted in some way to adjacent, undamaged leaves which are also relatively protected (Wratten et al., 1984; Edwards et al., 1985);

6. the changes in insect behaviour often correlate with measured chemical changes in the leaf (Tallamy, 1985; Chiang et al., 1987);

7. many of the documented cases of hebivores' moving following small meals concern conspicuous aposematic species; predator avoidance is less necessary in these groups, yet they still move away from damage (e.g. Edwards and Wanjura, 1989).

There is thus good evidence that wound-induced chemical changes are involved in the avoidance by insects of damaged leaves, though the behavioural basis of such avoidance is rarely studied. Such behavioural information is needed to help determine the significance of the insects' avoidance of damaged tissue for insect fitness.

INSECT BEHAVIOUR IN RELATION TO WOUND-INDUCED CHANGES IN LEAVES

Some herbivorous insects show elaborate and complex behavioural traits associated with feeding. Two examples are the trenching behaviour of *Epilachna borealis* (Coleoptera; Coccinellidae) adults and larvae in relation to induced cucurbitacin levels (Tallamy, 1985), and the cessation of feeding by some Lepidoptera and Hymenoptera which then sever the leaf at the petiole and in some cases gnaw the petiole stump (Edwards and Wanjura, 1989). A plausible explanation for such behaviour is that it is a way of avoiding or minimising the consequences of wound-induced changes in the foliage on which the insect has fed (Edwards and Wanjura, 1989) or on which it is about to feed (Tallamy, 1985). The evolution of the elaborate behaviour involving leaf removal after small amounts of feeding suggests very strong selective pressures on herbivores. Given these extreme behaviours which are seen in a few insects, it seems highly likely that other insects also have behavioural responses to wound-induced changes, although these may be less obvious and require novel and detailed

Table 1 The numbers and sizes of meals taken by larvae of *Orthosia stabilis* Dennis and Schiffermüller on previously-damaged and undamaged shoots of birch (*Betula pubescens* Ehrh.). Ten per cent of each leaf lamina was removed in the field using a hole punch. Twenty-four hours later, shoots were collected and two third-instar larvae were allowed to forage on each shoot for twenty-four hours. $\chi^2=19.9$; $p<0.001$

	Control	Damaged
No. small meals (<1% of leaf area)	21	55
No. large meals (>5% of leaf area)	90	61

methods to detect them. In recent work at Southampton we have begun to explore the consequences of small amounts of damage for insect foraging behaviour. The parameters we study include:

1. the duration of the feeding bout,
2. the number of feeding initiations,
3. the number of small meals compared with the number of large meals,
4. the distance moved between feeding bouts,
5. the size distribution of feeding initiations,
6. the proximity of feeding to initial damage.

Recent work (Table 1) has shown just how many initiations insects make, especially when feeding on foliage with small amounts of initial damage. The experiments which generated the data in Table 1 also involved time-lapse video recordings of foraging larvae and revealed a large number of initiations by these larvae which led to no substantial feeding, especially on the damaged leaves. On these leaves larvae fed chiefly from the edge and it was clear that in many cases the leaves were rejected after the larvae had taken only a very small amount from the tips of some of the leaf teeth. Also, a significantly higher proportion of previously damaged leaves received no grazing whatsoever, providing evidence for early rejection of leaves which was suspected to have occurred in the Silkstone (1987) fieldwork. Work in progress uses time-lapse video of insects on whole plants, cut shoots and on diets. In the latter case, artificial leaves made from diet and containing a range of levels of secondary plant compounds can be offered to larvae in a grid and the movements and 'giving-up times' of the foraging larvae can be recorded via the video.

CONSEQUENCES OF WOUND-INDUCED CHANGES FOR THE PLANT AND THE INSECT

These have been reviewed recently by Edwards et al. (1990). For the plant, the fact that the yougest leaves show the strongest wound-induced change following damage has the effect in some experimental systems of driving larvae away from the shoot tip. In plants which are competing for light, this deflection of grazing away from the fast-growing shoot-tips may confer upon the plant a higher competitive ability. However, there may also be consequences for insect fitness; a recently pub-

lished simulation model (Wratten et al., 1988) attempted to generate the patterns of movement of larvae on damaged and undamaged plants. It revealed possible adverse consequences for these herbivores in terms of a greater development time and a greater mortality rate associated with movement. In the model these effects lead to substantial reductions in herbivore populations over a relatively short, (i.e. within- generation) time period. Some recent experimental work has also shown that wound-induced changes in foliage quality can cause reductions in populations of phytophagous arthropods (Croft and Hoying, 1977; Karben and Carey, 1984; West, 1985; Faeth, 1985, 1986; Harrison and Karban, 1986). Possible reasons why some studies have led to no such reductions in population levels (such as that of Fowler and Lawton, 1985) are discussed in Edwards et al. (1990). More experimental data are certainly needed from the laboratory and the field to continue to explore the hypothesis that intra-specific competition amongst insect herbivores may be much more common than previously suspected. If it is a common phenomenon, and one which is mediated by a reduction in host plant quality following insect feeding, then the ideas of Dempster and Pollard (1981), Dempster (1983) concerning the 'ceiling model' of herbivore population dynamics will receive further support.

REFERENCES

Bergelson, J. M. and J. H. Lawton (1988): Does foliage damage influence predation on the insect herbivores of birch? *Ecology 69,* 434-445.

Chiang, H., D. M. Norris, A. Ciepiela, P. Shapiro and A. Oosterwyk (1987): Inducible versus constitutive pi 227686 soybean resistance to Mexican bean beetle. *J. Chem. Ecol. 13,* 741-749.

Croft, B. A. and S. A. Hoying (1977): Competitive displacement of *Panonychus ulmi* (Acarina: Tetranychidae) by *Aculus schlechhtendali* (Acarina: Eriophyidae) in apple orchards. *Can. Entomol. 109,* 1025-1034.

Croxford, A. C., P. J. Edwards and S. D. Wratten (1989): Temporal and spatial variation in palatability of soybean and cotton leaves following wounding. *Oecologia 79,* 520-525.

Dempster, J. P. (1983): The natural control of populations of butterflies and moths. *Biol. Rev. 58,* 461-481.

Dempster, J. P. and E. Pollard (1981): Fluctuations in resource availability and insect populations. *Oecologia 50,* 412-416.

Edwards, P. J. and S. D. Wratten (1983): Wound-induced defences in plants and their consequences for patterns of insect grazing. *Oecologia 59,* 88-93.

Edwards, P. J., S. D. Wratten and H. Cox (1985): Wound-induced changes in the acceptability of tomato to larvae of *Spodoptera littoralis*: a laboratory bioassay. *Ecol. Entomol. 10,* 155-158.

Edwards, P. J., S. D. Wratten and S. Greenwood (1986): Constitutive and induced defences against insect grazing in British trees. *Oecologia 69*, 316-319.

Edwards, P. J., S. D. Wratten and R. M. Gibberd (1990): The impact of inducible phytochemicals on grazing behaviour of insect herbivores. In: M. J. Raupp and D. Jr. Tallamy (eds), *Phytochemical Induction by Herbivores.* Wiley, New York.

Edwards, P. J. and W. J. Wanjura (1989): Eucalypt-feeding insects bite off more than they can chew: sabotage of induced defences. *Oikos 54,* 236-248.

Faeth, S. H. (1985): Host leaf selection by leaf miners: interactions among three trophic levels. *Ecology 66,* 870-875.

Faeth, S. H. (1986): Indirect interactions between temporally separated herbivores mediated by the host plant. *Ecology 67,* 479-494.

Fowler, S. V. and J. H. Lawton (1985): Rapidly induced defenses and talking trees: the devil's advocate position. *Am. Nat. 126,* 181-195.

Gibberd, R., P. J. Edwards and S. D. Wratten (1988): Wound-induced changes in the acceptability of tree-foliage to Lepidoptera: within-leaf effects. *Oikos 51,* 43-47.

Hairston, N. G., F. E. Smith and L. B. Slobodkin (1960): Community structure, population control and competition. *Am. Nat. 44,* 421-425.

Harrison, S. and R. Karban (1986): Effects of an early-season folivorous moth on the success of a later-season species, mediated by a change in the quality of the shared host plant, *Lupinus arboreus. Oecologia 69,* 354-359.

Heinrich, B. (1979): Foraging strategies of caterpillars. Leaf damage and possible predator avoidance strategies. *Oecologia 42,* 325-337.

Karban, R. and J. R. Carey (1984): Induced resistance of cotton seedlings to mites. *Science 225,* 53-54.

Silkstone, B. E. (1987): The consequences of leaf damage for subsequent insect grazing on birch (*Betula* spp.): a field experiment. *Oecologia 74,* 149-152.

Strong, D. R., J. H. Lawton and T. R. E. Southwood (1984): *Insects on Plants. Community Patterns and Mechanisms.* Blackwell, Oxford.

Tallamy, D. (1985): Squash beetle trenching behaviour: an adaptation against induced cucurbit defenses. *Ecology 66,* 1574-1579.

West, C. (1985): Factors underlying the late seasonal appearance of the lepidopterous leaf-mining guild on oak. *Ecol. Entomol. 10,* 111-120.

Wratten, S. D., P. J. Edwards and I. Dunn (1984): Wound-induced changes in the palatability of *Betula pubescens* and *B. pendula. Oecologia 61,* 372-375.

Wratten, S. D., P. J. Edwards and L. Winder (1988): Insect herbivory in relation to dynamic changes in host plant quality. *Biol. J. Linn. Soc. 35,* 339-350.

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STRUCTURAL DAMAGE TO OAK LEAVES ALTERS NATURAL ENEMY ATTACK ON THE LEAFMINER, *CAMERARIA* SP. NOV. (DAVIS)

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ABSTRACT

Emory oak trees in central Arizona show widely varying degrees of resistance to attack by a dominant leafmining species, *Cameraria* sp. nov. Distribution of larvae of the leafminer is higly clumped among trees, within trees (on shade leaves), among leaves (on large leaves), and within leaves (on the proximal 1/2 of the leaf). Clumping is particularly perplexing because survival of co-occurring larvae is reduced via cannibalism. Those larvae surviving in multiply-mined leaves have reduced body size and thus fecundity. Variation in either nutritional or "defensive" chemistry does not explain variation in leafminer distribution or survival at various spatial scales. Experiments indicate that distribution of suitably-sized leaves influences initial colonization, aggregation, and subsequent survival of leafminers. Mean leaf size is a good predictor of tree resistance.

Key words: *Cameraria*, folivory, induced responses, leafminers, natural enemies, parasitoids, predators, structural damage

INTRODUCTION

Changes in plants following folivory have been proposed as defenses against herbivores by inhibiting their growth and development and reducing fecundity (Fowler and Lawton, 1985; Raupp and Tallamy, 1989). Physical and chemical changes in plants induced by folivores may also indirectly affect herbivores by altering attack rates of natural enemies (Price, 1986; Price et al., 1980; Niemelä and Tuomi, 1987). The "induced defense via natural enemies" hypothesis (IDNE) proposes that induced changes increase natural enemy attack by 1) slowing development of insects so they are exposed longer to natural enemies, 2) increasing herbivore movement so that insects are more apparent to natural enemies, or 3) providing chemical or structural cues for natural enemies to locate insects (Heinrich and Collins, 1983; Vinson et al., 1987).

Despite its intuitive appeal, current support for the IDNE hypothesis is scarce. For example, Bergelson et al. (1986) and Bergelson and Lawton (1988) found that artificial leaf damage increased movement of casebearing caterpillars on birch, but increased predation did not ensue. Similarly, Hawkins (1988) showed that attendant damage to birch leaves did not increase parasitism of the leafminer, *Coleophora*

serratella. However, Faeth (1985, 1986) showed that collateral damage to oak leaves increased rates of parasitism for the leafminer, *Cameraria* sp. nov., as well as death from premature leaf abscission.

I test the IDNE hypothesis here via a field experiment where structural changes in leaves induced by folivory are simulated but chemical changes are controlled. This experiment is the reciprocal of Faeth and Bultman's (1986) experiment where chemical induction (increased tannins) was mimicked while controlling for structural cues that might be used by natural enemies to locate leafminers.

MATERIAL AND METHODS

Study system. *Cameraria* sp. nov. is a monophagous, univoltine leafminer of *Quercus emoryi*. Adults emerge from puparia within leaves in the spring as leaves are abscised, mate and females oviposit on newly expanded leaves. Eight larval instars feed within leaves from April or May to the following spring. Further details of the biology of the leafminer and oak is in Faeth (1986).

Field Experiment. To test the effect of physical changes in leaves on natural enemy attack, undamaged and unmined oak leaves were collected at Oak Flat, Arizona on 2 June 1988. One-half of the leaves were hole-punched (3 to 4 holes along one edge of each leaf). All the leaves were sprayed with Sprayon TFE ®. TFE is a long-lasting fluorocarbon telomer which dries on contact, is chemically inert, stable over a wide range of temperatures, and seals the leaf with a very thin coat. Structural differences between sealed undamaged and damaged leaves were thus preserved but any differences in induced volatile or contact chemicals were eliminated.

Sealed leaves were carefully tied with thread to the petiole of mined, but otherwise undamaged, leaves on four oak trees at Oak Flat on 7 June 1988. Sealed leaves overlapped the upper surface of the mined leaf but did not obscure the mine. I alternated tethering of control and experimental leaves as suitable leaves with viable second instar leafminers were encountered within trees. Sample sizes of both control and experimental leaves within trees were: Tree A, $n = 35$; Tree B, $n = 50$; Tree C, $n = 125$; Tree D, $n = 40$. Tethered leaves overlapped, and provided a continuous surface with mined leaves; a searching parasitoid landing on a sealed leaf could continue search on the mined leaf without flight. Similarly, sealed damaged leaves should present at least some of the same visual cues to predators as a mined leaf with folivore damage.

Control and experimental mined leaves were collected at the end of the mining period (20 March 1989) and returned to the laboratory. Some leaves could not be found and some were excluded from analyses if the tethered leaf was no longer overlapping the mined leaf. Final sample sizes for control and experimental leaves, respectively, were: Tree A, 24 and 22; Tree B, 35 and 35; Tree C, 107 and 83; Tree D, 29 and 29. Survival, incidences of attack by natural enemies, and death for other reasons were determined for leafminers by inspecting and dissecting each mine.

Further information on ascertaining specific causes of death is in Faeth (1986) and Faeth and Bultman (1986).

Cameraria is parasitized by more than 20 hymenopteran species; the majority of parasitism is caused by *Paraleurocerus bicoloripes* (Girault) (Hymenoptera: Encyrtidae), several species of *Sympiesis*: *S. conica* (Provancher), *S. stigmata* (Girault), and *S. acrobasidis* (Miller)), *Closterocerus trifasciatus* (Westwood), *C.* sp. near *cinctipennis* (Ashmead), *Pnigalio metacomet* (Crawford), and *Chrysonotomyia* sp. (Hymenoptera: Eulophidae) and *Chelonus* (*Microchelonus*) sp. and *Cotesia* sp. (Hymenoptera: Braconidae). The biology of the known species and that of species closely related to the unknown species indicates that all are generalist parasitoids of leafminers, barkminers, casebearers, gallformers, leaftiers, leafrollers, or strictly external-feeding insects. Most predation is caused by the arboreal ant, *Pseudomyrmex apache*, a generalist predator. Included in the predation category are incidences of host feeding by adult parasitoids. Parasitism and predation were considered as one category on statistical analyses because the question of interest is how natural enemies generally respond to structural damage.

RESULTS

Attack by natural enemies of leafminers with attached, damaged leaves was significantly greater than that of leafminers with attached, intact leaves (Table 1, Fig.

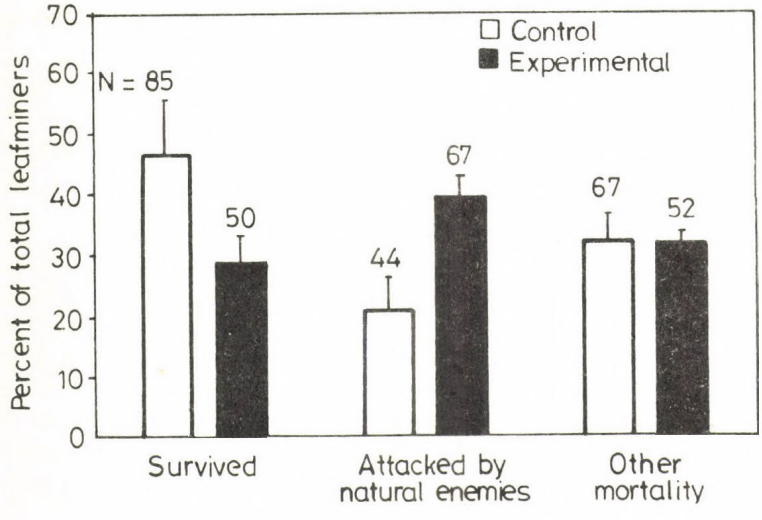

Fig. 1 Fates of leafminers in experimental (leaves with sealed, damaged leaves attached) and control (leaves with sealed, undamaged leaves attached). Bars are means and standard errors (with sample sizes above bars) of four trees. Untransformed percentages are shown; percentages were angularly transformed for ANOVA.

Table 1 Analyses of variance of rates of survival, death from natural enemies and other mortality (angularly-transformed) of leafminers in control and experimental leaves within four trees.

	Source of variation	Sum of squares	df	Mean square	F	p
Survived						
	Treatment	218.51	1	218.51	12.56	<0.05
	Error	104.34	6	17.39		
Natural Enemies						
	Treatment	269.58	1	269.58	31.27	<0.01
	Error	51.73	6.	8.62		
Other mortality						
	Treatment	0.38	1	0.38	0.08	>0.50
	Error	26.41	6	4.40		

1). Most of the mortality from natural enemy attack was from parasitoid attack (85.6% of total natural enemy attack) rather than from predation (14.4%). Mortality caused by factors other than natural enemies did not differ significantly between experimentals and controls (Table 1, Fig. 1).

Survival of leafminers with attached, damaged leaves was significantly less than those with attached, intact leaves (Table 1, Fig. 1). This decline in survival of leafminers tethered to damaged leaves appears to be due to the increase in attack by natural enemies, since other mortality factors do not vary between experimental and control leaves (Table 1, Fig. 1).

A preliminary experiment conducted in the previous season (1987-1988) corroborate these results. Parasitism rates were significantly greater for leafminers with attached, damaged leaves than those with attached, undamaged leaves (control, 21.7%, n = 143 mines; experimental, 35.2%, n = 108 mines, $\chi^2 = 5.63$, df = 1, $p<.05$). However, in this experiment, I used paraffin to seal leaves through which some volatile compounds may penetrate and some phenolic compounds can be found (D. W. Clark, pers. comm.), and the experiment was conducted within a single tree.

DISCUSSION

Physical damage as a consequence of previous folivory can alter attack by natural enemies on leafminers. Past studies of the impact of induced responses on the third trophic level, i.e., natural enemies, have produced conflicting results. Some show no effect (Fowler and MacGarvin, 1986; Bergelson et al., 1986; Bergelson and Lawton, 1988; West, 1985; Hawkins, 1988), while others show induced responses increasing natural enemy attack (Faeth, 1985, 1986). None of these studies, however, attempted to separate effects of chemical and structural changes in leaves that accompany folivory on natural enemy attack.

My results indicate that physical damage without induced chemical changes influence rates of parasitism. Previous experiments in this system demonstrated that

induced chemical changes (primarily tannins or tannin degradation products) also increased rates of parasitism attack (Faeth and Bultman, 1986). However, in that study overall survival was not altered; increases in parasitism were counterbalanced by decreases in other mortality. Elevated tannins may have protected larvae from entomopathogenic bacteria and fungi. Here, increases in attack by natural enemies resulted in lower survival, suggesting that physical damage by folivores can negatively influence population dynamics of *Cameraria* via changes in frequency of attack by the third trophic level.

If damage-mediated attack by natural enemies influences population dynamics of leafminers, then one would expect the leafminer to exhibit countermeasures. Indeed, this leafminer (Faeth, 1988) and others (Faeth 1985, 1986; Simberloff and Stiling, 1987) avoid ovipositing on folivore-damaged leaves. Although increased mortality due to premature leaf abscission (West, 1985; Faeth, 1987), reduced leaf area (Faeth, 1989), or direct effects of induced chemistry (West, 1985) can also decrease survival of leafminers feeding in folivore-damaged leaves, increases in natural enemy attack may serve to reinforce such behavior.

Hawkins (1988) warned that while the notion of parasitoid-mediated competition among folivores is intriguing, it is still largely uncorroborated. At least for *Cameraria*, structural changes due to folivory do appear to influence natural enemy attack and, consequently, survival. The parasitoids of *Cameraria* appear to be mostly generalists, attacking a variety of folivorous insects (only *P. bicoloripes* has exclusively leafmining or barkmining hosts) and therefore might be expected to respond to nonspecific leaf damage. Folivorous insect species with specialized natural enemies may be less affected by presence of other folivores if their natural enemies respond only to physical and chemical cues associated with the host itself and their species-specific feeding patterns.

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REFERENCES

Bergelson, J., S. Fowler, and S. Hartley (1986): The effects of foliage damage on casebearing moth larvae, *Coleophora serratella*, feeding on birch. *Ecol. Entomol. 11*, 241-250.

Bergelson, J. M. and J. H. Lawton (1988): Does foliar damage influence predation on the insect herbivores of birch? *Ecology 69*, 434-445.

Faeth, S. H. (1985): Host leaf selection by leaf miners: interactions among three trophic levels. *Ecology 66*, 870-875.

Faeth, S. H. (1986): Indirect interactions between temporally-separated herbivores mediated by the host plant. *Ecology 67*, 479-494.

Faeth, S. H. (1987): Community structure and folivorous insect outbreaks: the roles of vertical and horizontal interactions. In: P. Barbosa and J. C. Schultz (eds), *Insect Outbreaks*. Academic Press, New York.

Faeth, S. H. (1988): Plant-mediated interactions between seasonal herbivores: enough for evolution or coevolution? In: K. C. Spencer (ed), *Chemical Mediation of Coevolution*. Academic Press, New York. 391-414.

Faeth, S. H. (1989): Variable induced responses: Direct and indirect effects on oak folivores. In: M. J. Raupp and D. W. Tallamy (eds), *Phytochemical Induction by Herbivores*. John Wiley and Sons, New York. (in press).

Faeth, S. H., and T. L. Bultman (1986): Interacting effects of increased tannin levels on leaf-mining insects. *Entomol. exp. appl. 40*, 297-300.

Fowler, S. V. and J. H. Lawton (1985): Rapidly induced defences and talking trees: the devil's advocate position. *Am. Nat. 126*, 181-195.

Fowler, S. V. and M. MacGarvin (1986): The effects of leaf damage on the performance of insect herbivores on birch, *Betula pubescens. Oecologia 55*, 565-573.

Hawkins, B. A. (1988): Foliar damage, parasitoids, and indirect competition: a test using the herbivores of birch. *Ecol. Entomol. 13*, 301-308.

Heinrich, B. and S. L. Collins (1983): Caterpillar leaf damage and the game of hide-and-seek with birds. *Ecology 64*, 592-602.

Niemelä, P. and J. Toumi (1987): Does the leaf morphology of some plants mimic caterpillar damage? *Oikos 50*, 256-257.

Price, P. W., C. E. Bouton, P. Gross, B. A. McPheron, J. N. Thompson and A. E. Weis (1980): Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Ann. Rev. Ecol. Syst. 11*, 41-65.

Price, P. W. (1986): Ecological aspects of host plant resistance and biological control: Interactions among three trophic levels. In: D. J. Boethel and R. D. Eikenbary (eds), *Interactions of Plant Resistance and Parasitoid Predators of Insects*. Ellis Horwood, Chichester.

Raupp, M. J. and D. W. Tallamy (1989): *Phytochemical Induction by Herbivores*. Academic Press, New York. (in press).

Simberloff, D. and P. D. Stiling (1987): Larval dispersion and survivorship in a leaf-mining moth. *Ecology 68*, 1647-1657.

Vinson, S. B., G. W. Elzen and H. J. Williams (1987): The influence of volatile plant allelochemics on the third trophic level (parasitoids) and their herbivorous hosts. In: V. Labeyrie, G. Fabres and D. Lachaise (eds), *Insects-Plants*. Dr. W. Junk, Dordrecht. 109-114.

West, C. (1985): Factors underlying the late seasonal appearance of the lepidopterous leaf-mining guild on oak. *Ecol. Entomol. 10*, 111-120.

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PATHOGEN-HERBIVORE INTERACTIONS MEDIATED BY ACTIVATED RESISTANCE IN CUCUMBER

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ABSTRACT

Research with cucumber has shown that restricted (non-systemic) infection of a cotyledon or the first true leaf with certain fungi or viruses will induce persistent, systemic resistance to later challenges by a wide range of fungal, viral, and bacterial pathogens. We tested the hypothesis that restricted inoculation of cucumber with the anthracnose fungus *Colletotrichum lagenarium* or with tobacco necrosis virus would induce partial, systemic resistance to three generalist herbivores: the twospotted spider mite (*Tetranychus urticae*), the fall armyworm (*Spodoptera frugiperda*), and the greenhouse whitefly (*Trialeurodes vaporariorum*). We also studied the behavioral response of the cucurbit specialist *Diabrotica* to immunized plants. Results suggest that pathogen-activated induced resistance has little or no effect on plant suitability for herbivores. Similarly, wounding failed to induce systemic resistance to pathogens. Evidence for independent mechanism of induced resistance to pathogens or herbivores is discussed.

Key words: *Colletotrichum lagenarium, Tetranychus urticae, Spodoptera frugiperda, Diabrotica undecimpunctata, Trialeurodes vaporariorum,* cucumber, tobacco mosaic virus, pathogen-herbivore interaction, activated resistance, feeding preference, population growth, larval growth, oviposition.

INTRODUCTION

Immunization is the foundation of preventative medicine against infectious disease of humans and animals, but its potential as a means of protecting plants has only begun to be explored (McIntyre, 1980; Kogan and Paxton, 1983; Kuc, 1987). Experiments with cucurbits, tobacco, and other plants have shown that restricted (non-systemic) infection of a cotyledon or lower leaf with certain pathogens will induce persistent, systemic resistance to a wide range of fungal, viral, or bacterial agents. Immunization of cucumber, watermelon, or muskmelon with the anthracnose fungus *Colletotrichum lagenarium* (*C. lag.*) or tobacco necrosis virus (TNV) protects these plants against at least 13 unrelated diseases (Kuc, 1987). The mechanism involves a chemical signal, as yet uncharacterized, which is produced at the site of induction and systemically translocated, sensitizing the plant to respond rapidly to infectious agents. The response in cucurbits involves enhanced phytoalexin accumulation and lignification at the site of challenge, a 50-fold increase in chitinase, and two- to three-fold increases in B-glucanase and peroxidase (Boller, 1985; Kuc, 1987). Protection lasts at least 4-6 weeks, can be extended through fruiting with a booster

inoculation, and has been demonstrated in the field (Kuc, 1987). Activation of systemic resistance by simple chemical substances (Dubrava et al. 1988; Gottstein and Kuc, 1989) underscores the potential practicality of immunizing plants by field spraying or seed treatment.

There is some evidence that restricted infection with a plant pathogen can also induce partial, systemic resistance to herbivores. In separate experiments, immunization of tobacco with a non-systemic mosaic virus resulted in 11% reduction in reproduction of the aphid *Myzus persicae* and 16% reduction in growth rate of hornworms reared on systemically protected leaves (McIntyre, et al., 1981; Hare, 1983). Possible effects of pathogen-activated resistance on suitability of other plants for herbivores are unknown. We report here the results of ongoing experiments involving interactions between pathogen-activated resistance and arthropod herbivores of cucumber.

MATERIALS AND METHODS

Culture of host and pathogens. Cucumber plants, *Cucumis sativus* L. cv. Wisconsin SRM-58, a bitter variety, were grown in the greenhouse using methods outlined by Dean and Kuc (1986). Culture of *C. lag.* and TNV was as described by Dean and Kuc (1986) and Jenns and Kuc (1977), respectively.

Inoculations and challenges. Systemic resistance to pathogens was induced by rubbing the abaxial surface of leaf 1 with a cheesecloth pad dipped in TNV inoculum, which gave well-defined, local lesions within a few days. Plants were immunized when the first leaf was fully expanded and the second leaf was about two-thirds expanded. Since rubbing results in minor abrasion, we included controls rubbed with distilled water as well as non-rubbed controls. All herbivore experiments began 7 days after immunization. Induction of systemic resistance to pathogens was confirmed by excising leaf 4 at the start of each experiment. Excised leaves were placed in plastic boxes (30 × 40 cm) lined with moist germination paper, and challenged on the abaxial surface with thirty, 10 μl drops of a *C. lag.* conidial suspension (10^4 spores ml^{-1}) (Gottstein and Kuc, 1989). Number and diameter of fungal lesions were determined after 7 days.

Population growth of twospotted spider mite. Three teneral, mated female mites, *Tetranychus urticae* Koch were placed on the adaxial surface of leaf 3 of immunized or control plants and confined within a ring (5 cm diam.) of Tangle-trap^R adhesive. Such rings were found not to interfere with induced, systemic resistance to *C. lag.* within the enclosure (unpublished data), nor do they affect the suitability of cucumber leaves for mites (Gould, 1978). There were 10 single plant replicates per treatment; the experiment was repeated twice. Female survival was recorded daily; total progeny were counted after 8 or 9 days.

Fall armyworm growth and survival. Newly-molted second instar *Spodoptera frugiperda* (J. E. Smith) from a lab colony were held in individual petri dishes (15 cm) on moist filter paper and fed excised leaves from immunized or control plants. Larvae

Table 1 Systemic resistance to *Colletotrichum lagenarium* in cucumber induced by inoculation with tobacco necrosis virus, and lack of effect on mite population growth.

Treatment	*C. lagenarium* lesions (mm^2)	No. progeny per female per day	Total mite population
Experiment 1			
TNV	164±45 a	5.4±0.5 ab	193±17 a
Water	948±129 b	6.4±0.5 bc	203±14 a
Control	881±150 b	4.8±0.5 a	166±20 a
Experiment 2			
TNV	64±21 a	5.2±0.6 a	183±17 a
Water	736±97 b	4.7±0.5 a	168±20 a
Control	973±6.3 b	3.6±0.6 a	180±18 a

Table 2 Systemic resistance of cucumber to *Colletotrichum lagenarium* induced by inoculation with tobacco necrosis virus, and lack of effect on fall armyworm.

Treatment	*C. lagenarium* lesions (mm^2)	Larval weight gain (mg)	Days to pupation	Pupal weight (mg)
TNV	49±6 a	342±27 a	24.5±1.0 a	194±6 a
Water	450±33 b	318±26 a	25.3±0.9 a	197±7.6 a
Control	432±32 b	306±29 a	25.7±0.7 a	190±7.3 a

All data are means ±SEM. Means within a column followed by the same letter do not differ significantly (ANOVA followed by Tukey's test, $\alpha = 0.05$)

were initially provided the third leaf, followed by freshly-excised upper leaves at 4 day intervals until they had pupated. Larvae were weighed every 5 days; days to pupation and pupal weight were noted. There were 18 replicates per treatment, each consisting of a single larva fed foliage from a separate plant. Plants had grown 12 to 15 leaves by the end of the experiment. Removal of leaves above the inducer leaf does not reduce systemic protection of remaining upper leaves (Kuc, personal communication).

Feeding preference assays. Leaf discs (2 cm diam.) were cut from the third leaf of immunized or control plants and offered to newly-molted 4th instar fall armyworms or 3-4-day old adult spotted cucumber beetles, *Diabrotica undecimpunctata howardi* Barber in choice tests. Six discs (3/treatment) were arranged alternately on moist filter paper in petri dishes (10 cm). A single insect was placed in each dish and allowed to feed for 24 h. Consumption (mg dry weight) was determined gravimetrically from wet and dry weights of original and remaining leaf tissue, using representative discs from the same leaves as standards.

Oviposition tests with greenhouse whitefly. TNV-immunized and control plants were paired on the greenhouse bench and enclosed cages (60×45×45 cm) covered with fine organza screen. *Trialeurodes vaporariorum* (Westwood) were aspirated from tobacco plants and about 300 (mixed sex) were released into each cage. Leaf 3 was excised and challenged with *C. lag.* at the same time. Cages were removed after 96

h and oviposition response was determined by cutting eight discs (2 cm diameter) from leaf 4 of each plant and examining them with a binocular microscope. The experiment was repeated 3 times, with 10, 10, and 5 replicates, respectively.

RESULTS

Plants that had been immunized with TNV on leaf 1 showed significant systemic resistance to *C. lag.* when challenged after 7 days. The average reduction in the area of fungal necrosis relative to water-rubbed controls was 83 and 91%, respectively, in the two mite experiments (Table 1), which supports the earlier findings of Jenns and Kuc (1977). Rubbing itself did not reduce the number or size of necrotic lesions. There were no significant differences in mite population growth on TNV-immunized plants relative to water-rubbed or non-rubbed controls (Table 1). Similarly, we found no significant differences in larval weight gain, length of developmental period, or pupal weight of fall armyworms reared on foliage from TNV-immunized versus control plants, even though the expression of *C. lag.* symptoms was reduced by 89% on leaves from the same plants (Table 2).

Neither fall armyworms nor adult cucumber beetles discriminated between leaf discs from plants that had been immunized with TNV or control plants. Mean consumption (mg dry weight) by armyworms in the first trial was 1.5 ± 0.2 vs. 1.3 ± 0.1 ($t=0.68$, $P=0.53$, 4 df) and by cucumber beetles was 0.4 ± 0.04 vs. 0.4 ± 0.1 ($t=0.02$, $P=0.98$, 4 df), for TNV-immunized vs. controls, respectively. In the second trial with cucumber beetles, consumption averaged 0.5 ± 0.1 for both treatments. Reduction in area of fungal necrosis resulting from challenge with *C. lag.* averaged 97% on TNV-immunized plants.

Similarly, adult whiteflies did not discriminate between TNV-immunized and control plants for oviposition (number of eggs/mm^2 in the three trials averaged 9.8 ± 1.9 vs. 9.8 ± 1.2, 10.9 ± 1.5 vs. 10.6 ± 1.9, and 12.2 ± 4.8 vs. 11.8 ± 2.3 for immunized vs. control plants, respectively; all P-values ≥ 0.9). Reduction in area of fungal necrosis averaged 93, 84, and 55% for immunized plants relative to controls (all differences significant at $P \leq 0.001$), confirming induction of systemic resistance to *C. lag.*.

DISCUSSION AND CONCLUSIONS

The non-specificity of pathogen-activated antimicrobial defenses of plants has fueled speculation that immunization could provide the added benefit of suppression of arthropod pests in the field (see McIntyre, 1980; Kogan and Paxton, 1983; Hare 1983). As reported previously by Jenns and Kuc (1977), we found that immunization (i.e., restricted inoculation) of leaf 1 of cucumber with TNV provided consistent, systemic resistance to later challenge by the anthracnose fungus *C. lagenarium*. However, unlike earlier reports with tobacco, immunization of cucumber with this non-systemic virus neither increased nor decreased susceptibility or palatability of upper

leaves to arthropod herbivores. We have obtained similar results in other experiments in which *C. lag.* was used as the inducing agent (Ajlan, unpublished data). The general nature of the pathogen-activated resistance response in cucumber apparently does not extend to arthropod herbivores.

Wounding of leaves of squash or zucchini has been implicated in induction of cucurbitacins and shifts in the suitability of non-damaged leaves for herbivores (Tallamy, 1985), although in related work we were unable to demonstrate systemic effects of wounding on palatability of cucumber to fall armyworms (a generalist) or to *Diabrotica*, which responded positively to similar wounding of zucchini (Tallamy, 1985). Neither wounding nor previous feeding by mites was found to induce resistance to *C. lag.* The mechanisms and specificity of activated resistance in cucurbits may differ in response to induction by phytopathogens or herbivory.

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REFERENCES

Boller, T. (1985): Induction of hydrolases as a defense reaction against pathogens. In: J. Key and J. Kosuge (eds), *Cellular and Molecular Biology of Plant Stress*. A. Liss, New York. 247-262.

Dean, R. and J. Kuc (1986): Induced systemic protection in cucumber: time of production and movement of the signal. *Phytopathology 76,* 966-970.

Dubrava, N., R. Dean, and J. Kuc (1988): Induction of systemic resistance to anthracnose caused by *Colletotrichum lagenarium* in cucumber by oxalate and extracts from spinach and rhubarb leaves. *Physiol. Mol. Plant Pathol. 33,* 69-79.

Gottstein, H. and J. Kuc (1989): Induction of systemic resistance to anthracnose in cucumber by phosphates. *Phytopathology 79,* 176-179.

Gould, F. (1978): Resistance of cucumber varieties to *Tetranychus urticae*: genetic and environmental determinants. *J. econ. Entomol. 71,* 680-683.

Hare, J. (1983): Manipulation of host suitability for herbivore pest management. In: R. Denno and M. McClure (eds), *Variable Plants and Herbivores in Natural and Managed Systems*. Academic Press, New York. 665-680.

Jenns, A. and J. Kuc (1977): Localized infection with tobacco necrosis virus protects cucumber against *Colletotrichum lagenarium. Physiol. Plant Pathol. 11,* 207- 212.

Kogan, M. and J. Paxton (1983): Natural inducers of plant resistance to insects. In: P. Hedin (ed), *Plant Resistance to Insects*. Amer. Chem. Soc. Symp. Series, Washington, D.C. 153-171.

Kuc, J. (1987): Plant immunization and its applicability for disease control. In: I. Chet (ed), *Innovative Approaches to Plant Disease Control*. J. Wiley & Sons, New York. 255-273.

McIntyre, J. (1980): Defenses triggered by previous invaders: nematodes and insects. In: J. Horsfall and E. Cowling (eds), *Plant Disease: An Advanced Treatise. Vol. V. How Plants Defend Themselves.* Academic Press, New York.

McIntyre, J., J. Dodds, and J. Hare (1981): Effects of localized infection of *Nicotiana tabacum* by a tobacco mosaic virus on systemic resistance against diverse pathogens and an insect. *Phytopathology 71,* 297-301.

Tallamy, D. (1985): Squash beetle feeding behavior: an adaptation against induced cucurbit defenses. *Ecology 66,* 1574-1579.

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PLANT CHEMICALS INFLUENCING HOST PLANT SPECIFICITY IN *CEUTORHYNCHUS* SPECIES FEEDING ON CRUCIFERAE

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ABSTRACT

Host plant relationships of two monophagous *Ceutorhynchus* species (Coleoptera: Curculionidae) were studied in the laboratory: *C. constrictus* feeding on garlic mustard (*Alliaria petiolata*), *C. inaffectatus* feeding on dame's violet (*Hesperis matronalis*). Both species were stimulated to feed by glucosinolates, but in *C. constrictus* this effect was difficult to demonstrate. Other still unidentified feeding stimulants were present in extracts from the host plants. The role of glucosinolates and other compounds in recognition of host plants by these weevils is discussed.

Key words: *Ceutorhynchus constrictus, C. inaffectatus, Alliaria petiolata, Hesperis matronalis,* Cruciferae, Coleoptera, Curculionidae, host plant recognition, feeding stimulants, Gestalt, plant extracts, glucosinolates, sinigrin, glucotropaeolin, glucomatronalin.

INTRODUCTION

Many crucifer specialist insects are stimulated to feed or oviposit by glucosinolates (Fig. 1) (Chew, 1988; Nielsen, 1988; Städler, 1986 and references therein). Glucosinolates form a homogenous group of sulphur containing glucosides which are characteristic of the plant family Brassicaceae (Cruciferae) and a few other families (Fenwick et al., 1983). By enzymatic reactions in the plants the glucosinolates may be hydrolysed to simpler compounds, e.g. isothiocyanates, thiocyanates or nitriles (Fenwick et al., 1983). Volatile isothiocyanates are known as attractants for several crucifer specialist insects, but they are usually inactive or less active than the non-volatile glucosinolates in stimulating feeding and oviposition (Nair and McEwen, 1976; Tanton, 1965).

The specific responses to glucosinolates might allow the insects to recognize plants belonging to the Cruciferae among other plants, which do not contain these compounds. But crucifer specialist insects are able to discriminate between different members of the crucifer family, and this discrimination may depend on responses to other compounds than the glucosinolates. Glucosinolates from several non-host plants were as potent feeding stimulants as glucosinolates from host plants to two *Phyllotreta* species (Coleoptera: Chrysomelidae) (Nielsen, 1978; Nielsen et al.,

1979a). Similar results were obtained with *Ceutorhynchus assimilis Payk.* (Coleoptera: Curculionidae) (Larsen et al., 1985). The presence of other feeding and oviposition stimulants than glucosinolates in the host plants has been demonstrated by Nielsen et al. (1979b) and Schöni et al. (1987) and two of these compounds have been identified (Nielsen et al., 1979b).

These findings support the idea that insects perceive and respond to characteristic mixtures of compounds forming a stimulatory blend or a chemical Gestalt which is typical for the host plant(s) (Dethier, 1982). The number of compounds contributing to a stimulatory blend is unknown and may be different in different insects. Monophagous insects seemed to be particularly suitable for studying these aspects because they are supposed to perceive sharp "images" of their host plants (Schneider, 1987), and because all important compounds are supposed to be present in a single plant species. The genus *Ceutorhynchus* (Coleoptera: Curculionidae) is especially rich in monophagous species (Dieckmann, 1972). The present study includes two species which are reported to utilize only a single host plant in large parts of Europe: *C. constrictus* Marsh. feeding on *Alliaria petiolata* (Bieb.) Cavara and Grande (garlic mustard) and *C. inaffectatus* Gyll. feeding on *Hesperis matronalis* L. (dame's violet).

METHODS AND MATERIALS

Plants and beetles were collected at natural sites. Extracts were made from stem leaves of the two host plants with boiling 70% methanol. The 70% methanol extract was evaporated to a small volume and separated between water and chloroform in a separation funnel. The water phase (Fraction 1) containing most of the biological activity was further separated on a series of ion-exchange columns in capillary pipettes and four groups (fractions) were obtained:

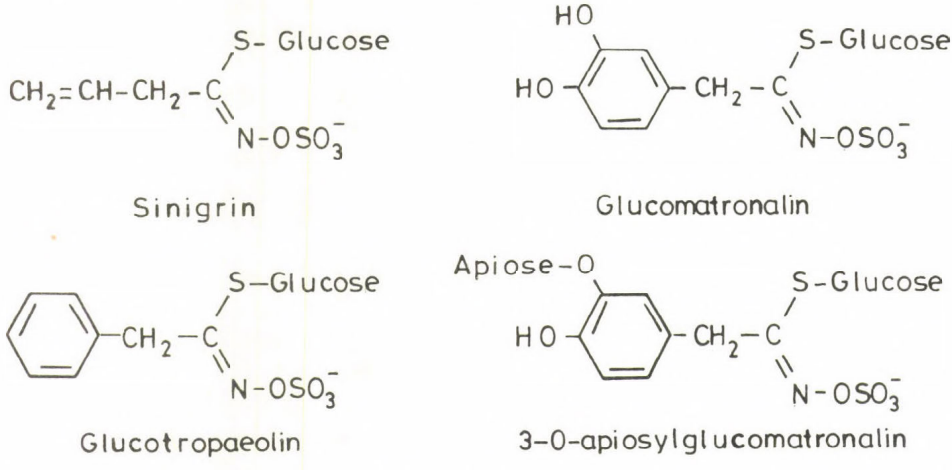

Fig. 1 Structures of some glucosinolates from *Alliaria petiolata* and *Hesperis matronalis*

Table 1 Feeding responses of two *Ceutorhynchus* species on pea leaf discs treated with various fractions containing the water soluble compounds from their host plants.

Fraction	Typical content	Stimulatory activity	
1.1	uncharged compounds e.g. sugars and flavonoids	+++[a]	0[b]
1.2	strong cations e.g. metal ions and amines	0	0
1.3	weaker cations e.g. most amino acids	0	0
1.4	anions e.g. glucosinolates	(+)	+++

[a] from leaves of *Alliaria petiolata* towards *C. constrictus*
[b] from leaves of *Hesperis matronalis* towards *C. inaffectatus*

1) neutral compounds,
2) strong cations,
3) weak cations, and
4) anions (Table 1).

Details of the extraction and separation procedure are given by Nielsen et al. (1989). Prior to bioassays the fractions were dissolved in water (1 ml per 0.1 g dry leaf material).

A leaf disc test was used to measure the feeding stimulatory activity to *C. constrictus*. Leaf discs of pea were dipped in a solution of the test compound or fraction (test discs) and presented to the weevils together with leaf discs treated with the solvent (control discs) (Nielsen et al., 1989). A similar assay was used for *C. inaffectatus*, but pieces of pea stem (pod attraps) were used instead of pea leaf discs (Larsen et al., 1985). Experiments lasted 24-40 hours. The feeding stimulatory activity was then measured by comparing the amount eaten on test and control treatments.

RESULTS AND DISCUSSION

Both monophagous species were stimulated to feed by the 70% methanol extracts from their respective host plants and the stimulatory compounds were found to be mainly water soluble (Nielsen et al., 1989, and in prep.). When the water soluble compounds were separated into four fractions, characteristic differences were found between the two monophagous species (Table 1). The major stimulatory activity towards *C. constrictus* was found in Fraction 1.1 from *Alliaria petiolata*. This fraction contained the uncharged compounds and very little activity was found in Fraction 1.4 containing the glucosinolates. In contrast Fraction 1.4 from *Hesperis matronalis* was the only stimulatory fraction for *C. inaffectatus* (Table 1).

The lack of a positive response to the glucosinolate fraction in *C. constrictus* was very surprising. The major glucosinolate in stem leaves of *A. petiolata* is sinigrin, while only trace amounts of glucotropaeolin (Fig. 1) are present (Nielsen et al., 1979a). Leaf discs of pea treated with a pure solution of sinigrin were also not fed upon by *C. constrictus* confirming the negative results obtained with the glucosinolate fraction from garlic mustard leaves (Nielsen et al., 1989). Sinigrin was then tested

in various combinations with other fractions and subfractions from garlic mustard leaves. In most of these tests, no effect of sinigrin could be demonstrated, but when combined with one particular subfraction of Fraction 1.1, a clear synergistic effect was found (Nielsen et al., 1989). Therefore, sinigrin is not a feeding stimulant when presented alone, but it contributes to a stimulatory blend found in garlic mustard leaves.

Efforts to identify the compounds which act synergistically with sinigrin have lead to the partial identification of a flavone glycoside. The compound is very similar and perhaps identical, to saponarin which has previously been tentatively identified from garlic mustard leaves (Paris and Delaveau, 1962), but a final structure elucidation has not yet been possible.

Although the flavone glycoside also seems to contribute to the stimulatory blend, mixtures of this compound and sinigrin are still much inferior to Fraction 1.1 in stimulating feeding in *C. constrictus*. Therefore, the stimulatory blend found in *A. petiolata* seems to contain more than these two compounds.

Only one of the four fractions obtained from *H. matronalis* leaves (Table 1) was stimulatory to *C. inaffectatus* when presented alone. This fraction (Fraction 1.4) contained the negatively charged compounds (anions), e.g. the glucosinolates. Leaves of *H. matronalis* turned out to contain a number of unidentified glucosinolates. The major constituents were glucomatronalin (3,4-dihydroxybenzylglucosinolate) and a 3-0-apiosyl derivative of this compound (Fig. 1) (Larsen et al., in prep.). The latter compound proved to be a very potent feeding stimulant for *C. inaffectatus* and so were a few other complex glucosinolates from *H. matronalis*. A number of simple glucosinolates without a glycosidic linkage in the side chain were much weaker stimulants (Larsen et al., in prep.). The complex glucosinolates from *H. matronalis* have not been reported from other plant species and the specific responses to these compounds might allow *C. inaffectatus* to recognize this plant among other crucifers.

A series of combination experiments were performed in order to examine whether other stimulatory compounds were present in *H. matronalis*. Until now one other fraction — a subfraction of Fraction 1.1 (Table 1) — has been found which stimulate feeding when presented together with the glucosinolates from *H. matronalis*. Fraction 1.1 was itself inactive probably because it contained both stimulatory and inhibitory compounds which were separated by the subsequent purification steps. The stimulatory activity from Fraction 1.1 could be traced through several purification steps, but the identity of the active compound(s) is still uncertain.

CONCLUSIONS

Feeding in both monophagous weevils is enhanced by different classes of compounds from their host plants including the glucosinolates. Therefore, host plant recognition seems to depend on responses to stimulatory blends and not only to single compounds from the host plants. The number of compounds contributing to these mixtures is unknown, but it seems to be possible to identify them one by one

from the extracts. Further studies should elucidate how compounds interact in the blend and whether it is possible to define a chemical Gestalt which would allow the insects to recognize their host plants.

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REFERENCES

Chew, F. S. (1988): Searching for defensive chemistry in the Cruciferae, or, Do glucosinolates always control interactions of Cruciferae with their potential herbivores and symbionts? No! In: K. C. Spencer (ed), *Chemical Mediation of Coevolution*. Plenum Press, New York. 81-112.

Dethier, V. G. (1982): Mechanism of host-plant recognition. *Entomol. exp. appl. 31,* 49-56.

Dieckmann, L. (1972): Beiträge zur Insektenfauna der DDR: Coleoptera-Curculionidae: Ceutorhynchinae. *Beitr. Entomol. 22,* 1-128.

Fenwick, G. R., R. K. Heaney and W. J. Mullin (1983): Glucosinolates and their breakdown products in food and food plants. *CRC crit. Rev. Fd. Sci. Nutr. 18,* 123-201.

Larsen, L. M., J. K. Nielsen, A. Plöger and H. Sørensen (1985): Responses of some beetle species to varieties of oilseed rape and to pure glucosinolates. In: H. Sørensen, (ed), *Advances in the Production and Utilization of Cruciferous Crops with Special Emphasis to Oilseed Rape*. Dr. W. Junk Publ., Dordrecht, Boston and Lancaster. 230-244.

Nair, K. S. S. and F. L. McEwen (1976): Host selection by the adult cabbage maggot, *Hylemya brassicae* (Diptera: Anthomyiidae): Effect of glucosinolates and common nutrients on oviposition. *Can. Entomol. 108,* 1021-1030.

Nielsen, J. K. (1978): Host plant selection of monophagous and oligophagous flea beetles feeding on crucifers. *Entomol. exp. appl. 24,* 562-569.

Nielsen, J. K. (1988): Crucifer-feeding Chrysomelidae: Mechanisms of host plant finding and acceptance. In: P. Jolivet, E. Petitpierre and T. H. Hsiao (eds), *Biology of Chrysomelidae*. Kluwer Akad. Publ., Dordrecht, Boston and Lancaster. 25-40.

Nielsen, J. K., L. Dalgaard, L. M. Larsen and H. Sørensen (1979a): Host plant selection of the horseradish flea beetle *Phyllotreta armoraciae* (Coleoptera: Chrysomelidae). Feeding responses to glucosinolates from several crucifers. *Entomol. exp. appl. 25,* 227-239.

Nielsen, J. K., L. M. Larsen and H. Sørensen (1979b): Host plant selection of the horseradish flea beetle *Phyllotreta armoraciae* (Coleoptera: Chrysomelidae): Identification of two flavonol glycosides stimulating feeding in combinaton with glucosinolates. *Entomol. exp. appl. 26,* 40-48.

Nielsen, J. K., A. H. Kirkeby-Thomsen and M. K. Petersen (1989): Host plant recognition in monophagous weevils. Specificity in feeding responses of *Ceutorhynchus constrictus* and the variable effect of sinigrin. *Entomol. exp. appl. 53,* 157-166.

Paris, R. R. ad P. Delaveau (1962): Isolement d'un nouvel heteroside flavonique. L'"alliaroside" des feuilles de l'*Alliaria officinalis* Andrz. (Cruciferae). *C. R. Acad. Sci. 254,* 928-929.

Schneider, D. (1987): Plant recognition by insects: A challenge for neuro-ethological research. In: V. Labeyrie, G. Fabres and D. Lachaise (eds), *Insects-Plants*. Dr. W. Junk Publ., Dordrecht. 117-123.

Schöni, R., E. Städler, J. A. A. Renwick and C. D. Radke (1987): Host and non-host plant chemicals influencing the oviposition behaviour of several herbivorous insects. In: V. Labeyrie, G. Fabres and D. Lachaise (eds), *Insects-Plants*. Dr. W. Junk. Publ., Dordrecht. 31-36.

Städler, E. (1986): Oviposition and feeding stimuli in leaf surface waxes. In: B. Juniper and R. Southwood (eds), *Insects and the Plant Surface*. Edward Arnold Publ., London. 105-121.

Tanton, M. T. (1965): Agar and chemostimulant concentrations and their effect on intake of synthetic food by larvae of the mustard beetle *Phaedon cochleariae* Fab. *Entomol. exp. appl.. 8,* 74-82.

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SEPARATE AND JOINT EFFECTS OF ROOT HERBIVORES, PLANT COMPETITION AND NITROGEN SHORTAGE ON RESOURCE ALLOCATION AND COMPONENTS OF REPRODUCTION IN *CENTAUREA MACULOSA* (COMPOSITAE)

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ABSTRACT

Potted rosettes of the biennial *Centaurea maculosa* (Compositae) were grown under 8 different stress treatments with each of the following factors being either present or absent: a) root herbivory by the moth *Agapeta zoegana* (Lep.: Cochylidae) and the weevil *Cyphocleonus achates* (Col.: Curculionidae), b) competition with the grass *Festuca pratensis* and c) nitrate shortage. We studied effects of these treatments on biomass allocation, concentrations of mobile and storage carbohydrates and nitrogen in aerial- and below-ground tissue, and on parameters of reproduction. Plants responded to the specific factors at different hierarchical levels, suggesting that the plant's potential to compensate varies greatly depending on the intensity, the pattern and timing of the stress applied. Competition with grass severely reduced both early and late components of plant fitness, i.e. parameters of growth and reproduction, respecively. Nitrogen shortage, applied as a single stress factor, had no effect on these parameters, except to reduce nitrogen concentration of leaves and seeds. The level of available nitrogen in the soil, however, seems greatly to influence the compensatory capacity of *C. maculosa*, when infested by herbivores. This produces effects from overcompensation through partial compensation up to probable damage by the herbivores to plants under nitrogen stress. Plants responded to root herbivory by accumulating carbohydrates in the leaves and increasing the allocation of nitrogen and biomass to the roots, thus adjusting the carbon/nutrient balance by restoring their optimal shoot/root ratio. These results support predictions of economic theory, that plants should adjust allocation so that all resources equally limit growth.

Key words: *Centaurea maculosa*, resource allocation, root herbivores, plant competition, nitrogen, multiple stress, compensatory growth, reproduction.

INTRODUCTION

The impact of root herbivores on plant fitness and on population and community structure has received very little attention by ecologists, despite the fact that they appear to have an important role (Andersen, 1987; Brown and Gange 1989). Data from diverse ecosystems indicate that more than 50% and up to 90% of the net primary production is commonly allocated to subterranean parts, of which up to 30% may be removed by subterranean herbivores in herbaceous plant communities (Bazzaz et al., 1987; Andersen, 1987). Subterranean herbivory was found to be more often chronic than acute, especially on plants, whose roots serve as storage organs and contain increased carbohydrate and nutrient concentrations (Andersen, 1987).

Three possible strategies appear to have evolved in plants to cope with herbivory:

1) chemical or other deterrents to feeding (e.g. Feeny, 1976; McKey, 1979),
2) compensatory growth through modification of the plant metabolism (McNaughton, 1983) and
3) an excess of functional capacity resulting in a degree of tolerance (Harper, 1977; Lovett-Doust, 1980; Andersen, 1987)

Chemical defence may be advantageous when herbivory is acute, but of unpredictable occurrence, while compensatory growth and tolerance can be assumed to be adaptations to environments with low levels of sustained herbivory. Root herbivores can disrupt the plant's functional integrity by reducing the absorption, accumulation and translocation of nutrients and by interfering with production of growth regulators (Brouwer 1983; Crawley, 1983; Andersen, 1987). Root herbivory generally leads to reduced shoot growth and compensatory root growth, thus adjusting for a favorable shoot/root ratio (McNaughton, 1983; Brouwer, 1983; Müller, 1989a). However, such compensation can be offset by the presence of additional stress factors (Bloom et al., 1985).

In a given environment, plant fitness can be expected to be maximized by optimizing the pattern of resource allocation to maintenance, growth, defence and reproduction (Hartnett and Abrahamson, 1979; Bazzaz et al., 1987). Relatively little is known about plant responses to multiple environmental stress, although most natural environments are stressful in several respects (Chapin et al., 1987). Hence, to understand the impact of herbivory on plant performance, additional interacting variables, such as the effect of neighbouring plants as well as the availability of water and nutrients for the plants have to be considered. An economic view using cost-benefit analysis and trade-offs in terms of the effects on overall plant performance as measured by growth and reproduction greatly improved the understanding of resource allocation patterns and of the plant's substantial homeostatic capacities for resource imbalances in the environment (Bloom et al., 1985; Chapin et al., 1987).

In this paper, we present some results of experiments with potted plants to study the separate and joint effects of 1) two root herbivores, the moth *Agapeta zoegana* (Lep.: Cochylidae) and the weevil *Cyphocleonus achates* (Col.: Curculionidae), 2) interspecific competition with the grass *Festuca pratensis*, and 3) nitrogen shortage, on the performance of the diploid monocarpic (normally biennial) *Centaurea maculosa* (Compositae). In particular, we investigated differences in the effect of intensity, timing and pattern between the three stress factors and their combinations on components of growth and reproduction, and analysed its underlying physiological mechanisms. The main goal of this study is to describe the average reaction norm of *C. maculosa* to root herbivory as modified by several other stress factors.

METHODS

Species. Centaurea maculosa ssp. *rhenana* (Boreau) is widely distributed in Europe (Dostal, 1976). In Eastern Europe, the species occurs in continental xeric plant

associations, but reaches its highest densities in its western distribution area at sites disturbed by human activities, such as ruderal sites or in abandoned or overgrazed pastures (Müller, 1989b). During surveys carried out in Central and Eastern Europe, we found 21 insect species associated with its roots, including the two specialist feeders *A. zoegana* and *C. achates* (Müller et al., 1989). Larvae of *A. zoegana* mine in the root cortex during autumn and spring, whereas the gall-inducing *C. achates* feeds in the central part of the tap-root and probably stresses the plant most severely during summer (Müller et al., 1988; Müller, 1989b). Both species were successfully established in North America for the biological control of *C. maculosa* (Müller and Schroeder, 1989). *Festuca pratensis* (Gramineae) is commonly associated with *C. maculosa* in its natural habitats in Europe and has a potential in reseeding programmes in North America as forage species and competitor of *C. maculosa*.

Experimental design and analysis. One-liter pots containing one *C. maculosa* rosette (grown from seeds collected from a field population in Eastern Austria in 1986 and sown in January 1987) were randomly assigned to one of 8 treatments resulting from a completely randomized 2x2x2 factorial design with either of the the three main factors being either present or absent: a) root herbivory by *A. zoegana* and *C. achates*, b) competition with the grass *F. pratensis*, and c) nitrogen shortage. Details of the treatments are given in Fig. 1. Each treatment was replicated 9-30 times, depending on the success of herbivore infestations. All plants produced seeds in 1988 and subsequently died. Half of the plants of each treatment were harvested on May 24, 1988, inspected for herbivory, separated into aerial- and subterranean tissue, freeze-dried and weighed. Carbohydrates (glucose, fructose, sucrose, starch and fructans) were analyzed using standard enzymatic methods (Bergmeyer, 1984) and a colorimetric assay specific for fructose (Nakamura, 1968). The nitrogen concentrations were assessed by a CHN analyzer (Haereus). In order to measure fecundity of the remaining plants, mature flower heads were regularly cut before they shed their seeds. On August 19, 1988, these plants were harvested, again separated into aerial- and subterranean tissue and oven dried at 65°C to save the seeds, but values were corrected to 65°C. The flower heads were dried at 40°C to determine dry weight allocation to reproduction. All seeds were then removed from the heads and the weight of a maximum of 50 randomly selected seeds per plant measured to estimate mean seed weight and the total number of seeds per plant. One sample per plant containing 3 randomly selected seeds was analysed by a CHN analyzer to assess the nitrogen concentration of the seeds.

Untransformed data were subjected to a series of one-way and 3-way analyses of variance to assess effects of treatments and factors, respectively. As the departures from normality of the residuals for this additive model were not large, significance tests should be valid (Sokal and Rohlf, 1981). A more detailed analysis of the data will be presented elsewhere.

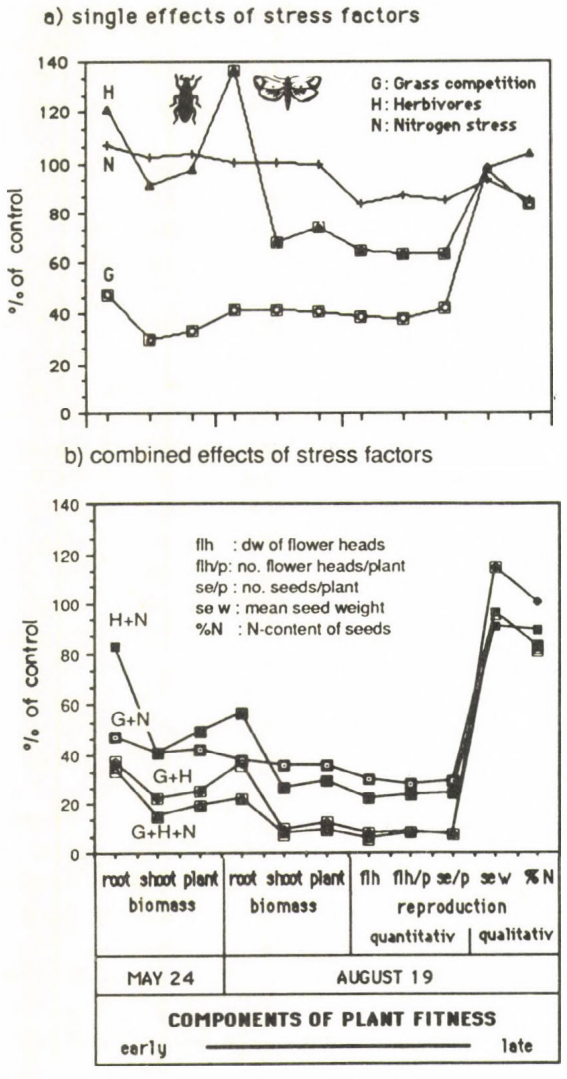

Fig. 1 Synopsis of separate and joint effects of root herbivory, plant competition and low nitrate availability on components of growth and reproduction in *C. maculosa*, expressed as percentage of controls (fertilized plants experiencing no herbivory and no competition). a) single effects of stress factors b) combined effects of stress factors. The factors are: Root herbivory - 5 first instar larvae of *A. zoegana* were transferred onto the rosettes between July 22 to August 8, and during September, the plants were exposed to one *C. achates* female until a maximum of 2 oviposition attemps were recorded; plant competition - 3 seedlings of *F. pratensis* were transplanted to each pot on April 22; nitrogen stress - controls were fertilized with 10 mg N/pot/week as $CaNO_3$ from April to July 1986 to reach nitrogen levels comparable to natural conditions (Ellenberg, 1977); stressed plants got the same amount of $CaCl_2$ to equalize Ca supply; each treatment was replicated 9-30 times; significantly different from the controls at the 5% level (Fisher PLSD)

Table 1 Chemical components of leaves and roots of *C.maculosa* grown under 8 different stress conditions and harvested on May 29, 1988 (cf. Fig. 1 for details of treatments)

Treatment	leaf starch (μg/mg dw)	root fructans (μg/mg dw)	leaf nitrogen (% of dw)	root nitrogen (% of dw)
Control	8.72	142.81	2.19	0.77
G: Grass competition	5.68	142.71	2.10	0.95
H: Herbivores	15.78	69.67*	2.07	1.20*
N: Nitrogen stress	9.09	130.13	2.04	0.89
G+H	11.92	71.44*	1.65*	1.26*
G+N	9.71	139.92	1.85*	1.05
H+N	14.05	92.44*	1.74*	1.14*
G+H+N	18.88*	92.66*	1.65*	1.41*

* Significantly different from controls at the 5% level (Fisher PLSD)

RESULTS

An overview of effects of the different combinations of stress factors on components of plant fitness is given in Fig. 1. As a single stress factor competition with grass reduced most severely both the aerial- and subterranean biomass. Surprisingly, low nitrogen availability in the soil showed no significant effect on biomass (Fig. 1a). In the absence of competition with grass, herbivores reduced shoot- and total biomass only under nitrogen stress (Fig. 1b, 2a). Roots probably compensated partially under nitrogen stress, but greatly overcompensated under good nutrient conditions (Fig. 2b). Competition with grass alleviated the stimulating effect of N-fertilization on root- and shoot-biomass of infested plants (Fig. 2). Multiple stress reduced biomass below the level achieved by each factor alone and effects were generally additive (Fig. 1b).

Herbivores greatly affected the plant's physiological parameters (Table 1). On the one hand starch concentrations in the leaves were significantly increased in plants infested by herbivores (3-way ANOVA) and the fructan concentration in the roots was reduced. On the other hand, the N-concentration of the leaves was significantly reduced, but that of the roots increased, as compared to controls. The high concentrations of fructans in the leaves (1.2-7.6% dw) did not vary between the treatments, but may be correlated with the bolting stage of plants at the time of harvest (Steinger and Müller, in prep). Nitrogen availability in the soil seems to have a central role as limiting factor, as the negative effect of the herbivores on the nitrogen concentration in the leaves was counterbalanced by N-fertilization. In the presence of competition with grass, however, N-fertilization had no effect on the nitrogen concentration of infested plants (Table 1). The herbivore impact on plant performance becomes evident when the shoot/root ratio is considered (Table 2). Herbivores strongly reduced the shoot/root ratio, especially at the end of the flowering period, when only the treatments involving herbivory had a significant effect (Table 2).

Table 2 Biomass allocation of *Centaurea maculosa* grown under 8 different stress conditions (cf. Fig. 1 for details of treatments)

Treatment	shoot ratio		flower heads (% of plant dw)
	May 24	Aug. 19	
Control	4.17	9.30	40.00
G: Grass competition	2.59*	9.12	37.17
H: Herbivores	2.93	4.50*	34.11
N: Nitrogen stress	3.89	9.06	32.99
G+H	2.52*	2.68*	28.28*
G+N	3.41	9.32	33.32
H+N	2.12*	4.77*	28.45*
G+H	1.68*	3.23*	25.17*

* Significally different from controls at the 5% level (Fischer PLSD)

Control plants invested 40% of their biomass in flower heads and seeds. Both nitrogen stress and herbivory significantly reduced allocation to reproductive tissues. Plants under maximum stress allocated only 25% of their biomass to reproduction. All treatments significantly reduced the number of flower heads and seeds per plant, with the effect of competition by grass being most pronounced (Fig. 1). Mean seed weight was only susceptible to nitrogen stress. Surprisingly, the combined effect of competition and herbivory was found to reduce greatly the number of seeds per plant, but to increase slightly the mean seed weight (Fig. 1). Additional nitrogen stress, however, reduced the latter by 17%. Grass competion and nitrogen stress significantly lowered the nitrogen content of the seeds, but this parameter was significantly increased by the root herbivores (3-factorial ANOVA). At maximum stress level the nitrogen concentration of the seeds reached only 82% of the controls.

DISCUSSION

The potential of a species to compensate for specific stress factors may shed light on a plant's evolutionary history, as a species is assumed to have optimized its resource allocation pattern with respect to the range of environments it most frequently encountered in the past.

The parameters measured in this study can roughly be arranged from early to late components of plant fitness (Fig. 1). Under the conditions studied, *C. maculosa* seems to be relatively susceptible to competition, as components of both growth and reproduction were greatly reduced in a grass neighborhood (Fig. 1). The fact that competition with grass reduced the shoot/root ratio (Table 2) in spring indicates that the effect of grass was mainly mediated through subterranean competition (Crawley, 1983; McNaughton, 1983) probably by depleting soil nutrients and water. Reduced total biomass may also have been due to the grass restricting the rooting zone available in the pots, i.e. to competition for subterranean space (Robbins and Pharr,

1988; Müller, 1989a). Nitrogen shortage, applied as a single treatment, surprisingly did not reduce biomass and reproductive parameters, except the N-concentration of the seeds. However, significant interaction terms between nitrogen stress and the other stress factors were found for most of the variates. Availability of nitrogen seems to influence greatly the compensatory capacity of *C. maculosa*, when infested by herbivores. Root herbivores stimulated overcompensation of root growth when nitrate was abundant, but only undercompensation was seen under nitrogen stress (Fig. 2). In this case, increased allocation of N to the roots subsequently also limited shoot

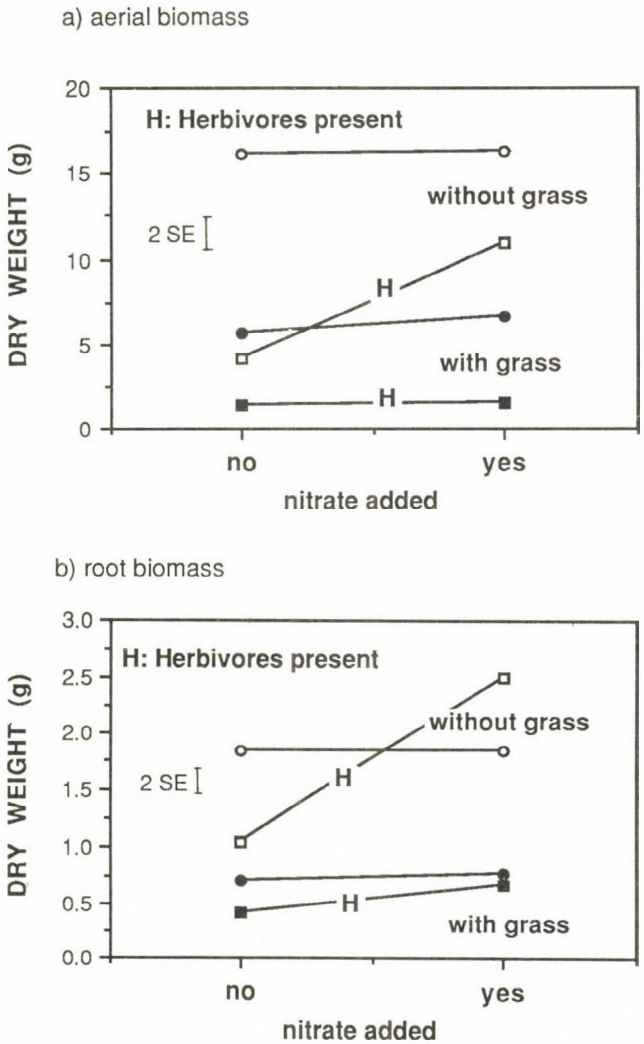

Fig. 2. *Biomass of C. maculosa* grown under 8 different stress conditions at the end of the growing season (August 19, 1988) (cf. Fig. 1 for details of treatment) a) aerial biomass b) root biomass

growth, resulting in an accumulation of starch in the leaves (Table 1), which is commonly observed in N-stressed plants (Rufty et al., 1988). Compensatory root growth took place partially at the expense of the stored fructans in the roots. Competition with grass depressed the compensatory growth induced by herbivores.

The application of economic theory to plant physiological ecology suggests that plants should adjust allocation so that all resources equally limit growth, i.e. by allocating new biomass to the acquisition of the resources that most strongly limit growth (Bloom et al., 1985). Our results seem to support this prediction. Nutrient stress, imposed indirectly by root herbivory, led to an accumulation of carbohydrates (starch) in the leaves. Plants responded by increasing allocation to root growth, thus adjusting the carbon/nutrient balance by restoring the optimal shoot/root ratio. Parameters of growth and reproduction can be considered as functional units, arranged in a hierarchical system (White, 1984; Marshall, 1989). Our study showed that the level at which the plant responded was dependent on the intensity, the pattern and the timing of stress applied. The consequences for the distribution of *C. maculosa* of such differential reactions to specific stress factors as well as more detailed analyses of these data and of parallel field experiments will be presented elsewhere. A better understanding of plant reactions to multiple stress and its consequences for population ecology will help to make more realistic predictions about the impact of biological control agents on weed populations.

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REFERENCES

Andersen, D. C. (1987): Below-ground herbivory in natural communities: a review emphasizing fossorial animals. *Quart. Rev. Biol. 62*, 261-286.

Bazzaz, F. A., N. R. Chiariello, P. D. Coley, and L. F. Pitelka (1987): Allocating resources to reproduction and defence. *BioScience 37*, 58-67.

Bergmeyer, H. U. (ed). (1984): *Methods of enzymatic analysis*. Verlag Chemie, Weinheim, Deer Field Beach, Fl, Basel, 3rd edition.

Bloom, A. J., F. S. Chapin III, and H. A. Mooney (1985): Resource limitation in plants - an economic analogy. *Ann. Rev. Ecol. Syst. 16*, 363-92.

Brouwer, R. (1983): Functional equilibrium: sense or nonsense? *Neth. J. Agric. Sci. 31*, 335-348.

Brown, V. K. and A. C. Gange (1989): Effects of root herbivory on vegetation dynamics. In: *Plant Root Systems: Their Effect on Ecosystem Composition and Structure*. Blackwell Scientific Publications. (in press)

Chapin III, F. S., A. J. Bloom, Ch. B. Field, and R. H. Waring (1987): Plant responses to multiple environmental factors. *BioScience 37*, 49-57.

Crawley, M. J. (1983): *Herbivory. The Dynamics of Animal-Plant Interactions*. Blackwell Publications, Oxford.

Dostal, J. (1976): *Centaurea* L. In: *Flora Europaea*. Cambridge Univ. Press, Vol. *4.*, 254-301.

Ellenberg, H. (1977): Stickstoff als Standortfaktor, insbesondere für mitteleuropäische Pflanzengesellschaften. *Oecol. Plant. 12*, 1-22.

Feeny, P. P. (1976): Plant apparency and chemical defence. In: J. Wallace, and R. L. Mansell (eds), *Biochemical Interactions between Plants and Insects*. Plenum Press, New York. 1-40.

Harper, J. L. (1977): *Population Biology of Plants*. Academic Press, London.

Hartnett, D. C. and W. G. Abrahamson (1979): The effects of stem gall insects on live history pattern in *Solidago canadensis. Ecology 60*, 910-917.

Lovett-Doust, J. (1980): Experimental manipulation of patterns of resource allocation in the growth cycle and reproduction of *Smyrnium olusatrum* L. *Biol. J. Linn. Soc. 13*, 155-166.

Marshall, D. L. (1989): Integration of response to defoliation within plants of two species of *Sesbania. Funct. Ecol. 3*, 207-214.

McKey, D. (1979): The distribution of secondary compounds within plants. In: G: A. Rosenthal, and D. H. Janzen (eds), *Herbivores. Their Interaction with Secondary Plant Metabolites*. Academic Press, New York. 55-133.

McNaughton, S. J. (1983): Compensatory plant growth as a response to herbivory. *Oikos 40*, 329-336.

Müller, H. (1989a): Growth pattern and effects on diploid and tetraploid spotted knapweed, *Centaurea maculosa* Lam. (Compositae) by the root-mining moth *Agapeta zoegana* L. (Lep.: Cochylidae). *Weed Res. 29*, 103-111.

Müller, H. (1989b): Structural analysis of the phytophagous insect guilds associated with the roots of *Centaurea maculosa* Lam., *C. diffusa* Lam. and *C. vallesiaca* Jordan in Europe: 1. Field observations. *Oecologia 78*, 41-52.

Müller, H. and D. Schroeder (1989): The biological control of diffuse and spotted knapweed in North America - what did we learn? *Proc. Knapweed Symp.*, Bozeman, Montana. (in press)

Müller, H, D. Schroeder and A. Gassmann (1988): *Agapeta zoegana* (L.) (Lep.: Cochylidae), a suitable prospect for the biological control of spotted and diffuse knapweed, *Centaurea maculosa* Lam. and *C. diffusa* Lam. (Compositae) in North America. *Can. Entomol. 120*, 109-124.

Müller, H., C. S. A. Stinson, K. Marquardt and D. Schroeder (1989): The entomofaunas of roots of *Centaurea maculosa* Lam., *C. diffusa* Lam. and *C. vallesiaca* Jordan in Europe: Niche separation in space and time. *J. Appl. Entomol. 107*, 83-95.

Nakamura, M. (1968): Determination of fructose in the presence of large excess of glucose. *Agr. Biol. Chem. 32*, 701-706.

Robins, N. S. and D. M. Pharr (1988): Effect of restricted root growth on carbohydrate metabolism and whole plant growth of *Cucumis sativus* L. *Plant Physiol. 87*, 409-413.

Rufty, T. W. Jr, S. C. Huber and R. J. Volk (1988): Alterations in leaf carbohydrate metabolism in response to nitrogen stress. *Plant Physiol. 88*, 725-730.

Sokal, R. R. and F. J. Rohlf (1981): *Biometry*. W. H. Freeman, San Francisco, 2nd ed.

White, J. (1984): Plant metamerism. In: R. Dirzo, and J. Sarukhan (eds), *Perspectives in Plant Population Ecology*. Sinauer, Sunderland. 15-47.

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HERBIVOROUS INSECTS' ADAPTATION TO INDIVIDUALITY OF HOST- PLANTS: BUD GALL MIDGE ON SIBERIAN LARCH

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Key words: *Dasyneura rozkovi, Larix siberica, Tetrastychus chakasicus, Torymus issaevi,* gall, demes, individual tree, synchronism, competition.

Abstract

In South Siberia larch gall midge (*Dasyneura rozkovi* Mamaev et Nikolski) forms large artichoke-like galls on the buds of larch (*Larix siberica* Led.). The success of infestation deals with the physiological ability of bud meristemic tissue to promote growth of normal galls (Issaev et al., 1988). This ability varies from bud to bud and individual larches possess different but rather stable percent of susceptible buds. The ratio of resistant buds, which are unable to form normal galls, is the key factor affecting gall midge mortality. Thus, the major mortality factor on trees with susceptible buds is intraspecific competition during the last larval instar. In resistant buds the young larvae die on the bract scales before reaching the bud apexes or on the apexes when unable to initiate gall development. Young larvae also die in abortive galls that do not reach half of the size of normal galls.

Differences between larches also affect the ratio of the two chalcid parasites of the midge. The mortality of first and second instar larvae in abortive galls causes the death of *Tetrastychus chakasicus* which attacks the early instar midge larvae. The second chalcid *Torymus isaevi* oviposits on later instars avoiding high mortality in abortive galls.

The variability between individual host trees and limited dispersal of female midges force gall midge demes to adapt to the individual characteristics of their hosts. This initiated the selection of midge genotypes that reach the bud apexes synchronously with the critical period of development on that particular tree. This shift in larval development can be attained by changes in the emergence and activity thresholds of ovipositing females. Adaptations of this type may be surprisingly rapid. Thus, in 17 generations gall midge colonized the crowns of four larch species on experimental plantation and the dynamics of adult emergence was observed to differ between species. Adaptations to specific individual hosts have been reported for

scale insects (Edmunds and Alstad, 1978) and the observed shifts in *D. rozkovi* emergence indicate that these midges also form isolated demes adapted to individual host phenology.

REFERENCES

Edmunds, G. F. and D. N. Alstad (1978): Coevolution in insect herbivores and conifers. *Science 199,* 941-945.

Isaev, A. S., Yu. N. Baranchikov and V. S. Malutina (1988): The larch gall midge in seed orchards of South Siberia. In: A. A. Berryman (ed), *Dynamics of Forest Insect Populations*. Plenum, New York. 29-44.

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PHYSICAL ATTRIBUTES OF EUCALYPT LEAVES AND THE HOST RANGE OF CHRYSOMELID BEETLES

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ABSTRACT

Evidence was sought for species-specificity in eucalypt-feeding chrysomelids, and the observed preferences were correlated with leaf waxiness and toughness. The distribution of eleven species of adult chrysomelids was monitored throughout a season on eight species of eucalypts. All beetle species had more than 50% of their numbers restricted to two species of eucalypt. Four patterns emerged: (i) six chrysomelid species were associated with *E. viminalis* and *E. macarthurii*, (ii) two species with *E. bridgesiana* and *E. melliodora*, (iii) two species with *E. bicostata* and *E. bridgesiana*, and (iv) one species with *E. aggregata*. Leaf toughness was measured with a penetrometer, and the leaves of *E. pauciflora* were significantly tougher than those of other species. Only 2% of all beetles occurred on this species. The effect of leaf waxiness was measured with a tenaxometer, which recorded a beetle's ability to cling to different surfaces. Beetles of all species were unable to cling to leaves that are coated with a waxy bloom. Excluding *Chrysophtharta m-fuscum*, only 3% of beetles occurred on *E. bicostata*, the eucalypt with the most heavily glaucous leaves. *C. m-fuscum* has overcome the problems of waxy foliage by several adaptations which enable it to colonize a habitat virtually unavailable to most other species. This has resulted in strong species specificity, with 95% of *C. m-fuscum* restricted to the two most waxy species, *E. bicostata* and *E. bridgesiana.*

Key words: Chrysomelidae, *Eucalyptus,* host-specificity, leaf toughness, leaf waxes, *Paropsis, Chrysophtharta,* host range.

INTRODUCTION

In many species of chrysomelids, both larvae and adults feed on the foliage of the same plant species. This provides the opportunity for a higher degree of specialization between insect and host than may occur in insects where adults and larvae have different nutritional requirements (Strauss, 1988). In Australia, larvae and adults of many species of *Paropsis* and *Chrysophtharta* (Chrysomelidae) feed on the young foliage of *Eucalyptus*. Eucalypts are evergreen sclerophyllous trees that usually exist in stable associations of 2 to 4 species, rather than in monospecific stands (Pryor, 1959). Many insect groups, including chrysomelids, have been shown to exhibit host preferences within such associations of co-dominant eucalypts (Burdon and Chilvers, 1974; de Little and Madden, 1975; Morrow, 1977), and thus must discriminate between species on a local scale.

Leaves of different plant species differ in physical characteristics as well as chemical composition. Plant chemistry is generally considered to be the predominant factor influencing the feeding behaviour of phytophagous insects. Eucalypt leaves are rich in volatile oils, tannins and phenols (Macauley and Fox 1980; Simmons and Parsons, 1987), so it is almost certain that interspecific differences in secondary compounds will play a major role in determining the host range of eucalypt-feeding insects. However, if physical hurdles have to be overcome by insects before feeding can occur, then these may result in an effective first line of defence against phytophagous insects. Physical factors that may protect leaves from herbivores include waxes (e.g. Stork, 1980a), trichomes (e.g. Schillinger and Gallun, 1968), toughness (e.g. Tanton, 1962) and shape (e.g. Fox and Morrow, 1981).

It has been suggested that the waxy bloom on juvenile leaves of some eucalypt species deters colonization by adult chrysomelids (Edwards, 1982). This possibility was investigated further in the present study, together with the consideration that leaf toughness may also influence the host range of chrysomelids. Interspecific differences in the physical characteristics of leaves were compared with observed patterns of host specificity of a chrysomelid community in a mixed species plantation of young eucalypts.

METHODS

Host plant associations of chrysomelids. Seven surveys were made between November, 1987 and May, 1988 on two adjacent plots of eucalypts at Crookwell, New South Wales. One plot contained *Eucalyptus viminalis* (number of trees = 24), *E. aggregata* (n = 24), *E. macarthurii* (n = 21) and *E. bicostata* (n = 21), and the other *E. bridgesiana* (n = 23), *E. mannifera* (n = 21), *E. melliodora* (n = 23) and *E. pauciflora* (n = 17). Trees were 3 m apart and each plot measured 24 x 24 m. The trees were 3 years old, and the mean height of each species ranged from 93 cm to 214 cm. On each survey all adult chrysomelids of the 11 most abundant species were counted on every tree. Specimens of all described and undescribed species have been lodged with the Australian National Insect Collection in Canberra.

Leaf toughness and water content. A penetrometer similar to that described by Feeny (1970) was used to compare leaf toughness of the 8 species of eucalypts. The spindle of the penetrometer was flat ended with a diameter of 3 mm. Water was added at a constant flow rate to a plastic bottle placed on the spindle, and the weight that was required to penetrate the leaf was converted to pressure (g/m^2). A stem was selected from a tree of each species, and half of every leaf on the stem was used to measure toughness, and the other half to measure water content after 48 h at 60°C. For those species with paired leaves, only one leaf from each pair was measured. The foliage on each stem ranged from fully expanded old leaves to young partially expanded leaves. Young leaves with a width of less than 6 mm were too small to be tested for toughness. Surveys of leaf toughness were done in mid-summer (January) and in late autumn (May).

Table 1 Distribution of adult chrysomelid beetles in a plantation of 3-year old eucalypts at Crookwell, NSW. Results represent the totals of 7 surveys between November 1987 and May 1988. Groups of chrysomelid species showing similar host preferences are enclosed by dotted lines.

Chrysomelid species	Distribution of beetles on eucalypts (%)									Total
	E. vim	E. mac	E. mel	E. bri	E. bic	E. agg	E. man	E. pau	%	(n)
C. inconstans	48	29	1	8	1	9	2	2	100	(1413)
C. decolorata	29	23	6	9	1	9	19	4	100	(1090)
P. charybdis	34	42	0	4	4	12	2	2	100	(232)
P. porosa	19	38	2	19	11	8	2	1	100	(184)
C. nobilita	38	41	0	5	2	9	5	0	100	(155)
P. 'nr. delittlei'	14	49	0	11	7	9	7	3	100	(150)
C. variicollis	8	2	69	9	1	6	4	1	100	(211)
C. 'antique gold'	12	0	41	21	0	5	12	9	100	(77)
C. m-fuscum	1	2	1	65	30	0	1	0	100	(568)
P. aegrota	16	22	1	29	24	5	3	0	100	(276)
C. 'fluoro green'	5	2	0	1	0	91	1	0	100	(345)
	27	23	6	16	6	14	6	2	100	(4,701)

*E. vim = *E. viminalis*, E. mac = *E. macarthurii*, E. mel = *E. melliodora*, E. bri = *E. bridgesiana*, E. bic = *E. bicostata*, E. agg = *E. aggregata*, E. man = *E. mannifera*, E. pau = *pauciflora*.

Effect of leaf waxes on beetle adhesion. A tenaxometer similar to that described by Stork (1980b) was used to measure the force with which adult chrysomelids pulled across the surface of eucalypt leaves. The tenaxometer consisted of a Harvard® isometric force transducer (0-50 g) coupled to a Tolshin Electron®chart recorder (1 mv - 2 v). A wire glued with Bostik® to the back of each beetle was attached to the force transducer by a cotton thread. A beetle was placed on a horizontal leaf and allowed to pull at right angles to the force transducer until consistent peaks were obtained on the chart recorder. The highest peak of each trace was converted to mass pulled. Eight males of *Chrysophtharta m-fuscum, C. variicollis, Paropsis charybdis* and *P. 'nr. delittlei'* were weighed and tested on two eucalypts with non-waxy leaves (*E. pauciflora, E aggregata*) and two with waxy leaves (*E. bridgesiana, E. bicostata*).

Rearing trials. The two chrysomelid species that showed most host specificity in the field (*C. m-fuscum* and *C. 'fluoro green'*) were reared on all eight foliage types in the laboratory at 23°C : 15°C, 12hL : 12hD. Eggs were collected in the field and two replicates of ten newly-hatched *C. m-fuscum* larvae and three replicates of 10 *C. 'fluoro green'* larvae were caged on sprigs of fresh foliage. The foliage stem was placed in water and the foliage replaced twice a week. Survival and duration of development to the prepupal stage, and prepupal weight were used to compare the suitability of each foliage type for the two chrysomelid species.

Table 2 Leaf toughness of eight species of eucalypts at two times of the year, measured on the leaf with the highest water content on each branch.

Species	Leaf toughness (gmm^{-2})		
	summer	autumn	mean
E. pauciflora	82.7	97.9	90.3
E. bridgesiana	62.4	66.9	64.7
E. melliodora	40.0	71.3	55.7
E. aggregata	60.0	47.2	53.6
E. bicostata	49.5	50.9	50.2
E. mannifera	41.2	59.3	50.3
E. viminalis	45.5	39.3	42.4
E. macarthurii	28.4	31.3	29.9

RESULTS

Host plant associations of chrysomelids. Almost 5,000 chrysomelid beetles were counted during the surveys, and four patterns of host-plant associations were recognized (Table 1). The first six chrysomelid species listed in Table 1 had more than 50% of individuals on *E. viminalis* and *E. macarthurii.*

More than 50% of *C. variicollis* and *C. 'antique gold'* were found on *E. melliodora* and *E. bridgesiana*, with the former eucalypt species being particularly favoured (69%) by *C. variicollis*.

C. m-fuscum and *P. aegrota* had more than 50% of individuals on *E. bridgesiana* and *E. bicostata*, the two eucalypts with a conspicuous waxy bloom on their leaves. This association was particularly strong for *C. m-fuscum*, with 95% of all beetles being recorded on the two waxy-leafed eucalypts. Of the other nine chrysomelid species, only 3% of individuals were found on the two waxy-leafed eucalypts, and in fact six species had two or fewer individuals on the eucalypt with the densest waxy bloom, *E. bicostata*.

C. 'fluoro green' showed the strongest host preference of all the chrysomelids, with 91% being found on *E. aggregata*. In general, *E. aggregata* was avoided by the other chrysomelids, with less than 2% of individuals being recorded on this species. Overall, the eucalypt on which the lowest number of beetles was recorded was *E. pauciflora*, which has thick leathery leaves.

Leaf toughness and water content. Leaf toughness increased and water content decreased as leaves aged, that is in leaves more distant from the shoot tip. This relationship occurred for all species, and is shown in Fig. 1 for a species with tough leaves (*E. pauciflora*), a species with intermediate leaves (*E. bicostata*) and a species with soft leaves (*E. viminalis*). In summer, in all species except *E. macarthurii* and *E. mannifera*, there was a slight decrease in water content in the very youngest leaves (Fig. 1 - summer). These leaves were very small and presumably contained a high ratio of cell wall to cell contents. In autumn there was no decrease in water content of the youngest leaves (Fig. 1 - autumn), except in *E. aggregata* and *E. bridgesiana.*

Table 3 The ability of male chrysomelid beetles to cling to different *Eucalyptus* leaves. Results are expressed as weight pulled in grams.

Chrysomelid species (n)	Weight of beetles mg (X±SD)	Weight (g) pulled on non-waxy foliage		Weight (g) pulled on waxy foliage	
		E. pauciflora	*E. aggregata*	*E. bridgesiana*	*E. bicostata*
C. m-fuscum (8)	29.34± *3.96*	2.33±*0.55*	2.12±*0.55*	0.79±0.38	0.04±0.05
C. variicollis (8)	57.11±7.70	3.60±0.66	3.73±0.79	0.57±0.25	0.15±0.05
P. 'nr. delittlei' (8)	129.50±20.42	5.71±1.76	4.75±1.66	0.76±0.27	0.40±0.45
P. charybdis (8)	134.23±12.00	6.38±0.63	6.03±1.03	1.57±0.95	0.35±0.44

Presumably in autumn the youngest leaves on each stem were more mature than the youngest leaves on stems collected in summer.

The correlation between leaf toughness and water content, and the seasonal change in this relationship made it difficult to compare directly leaf toughness between species. For those stems that showed a decrease in water content in the youngest leaves (Fig. 1 - summer), the leaf with the highest water content was selected to compare toughness between species. It was considered that this leaf represented a comparable stage of development for each species, and was well within the range of leaves eaten by chrysomelid adults and larvae. For those stems that did not show a decrease in water content in the youngest foliage (Fig. 1 - autumn), the youngest

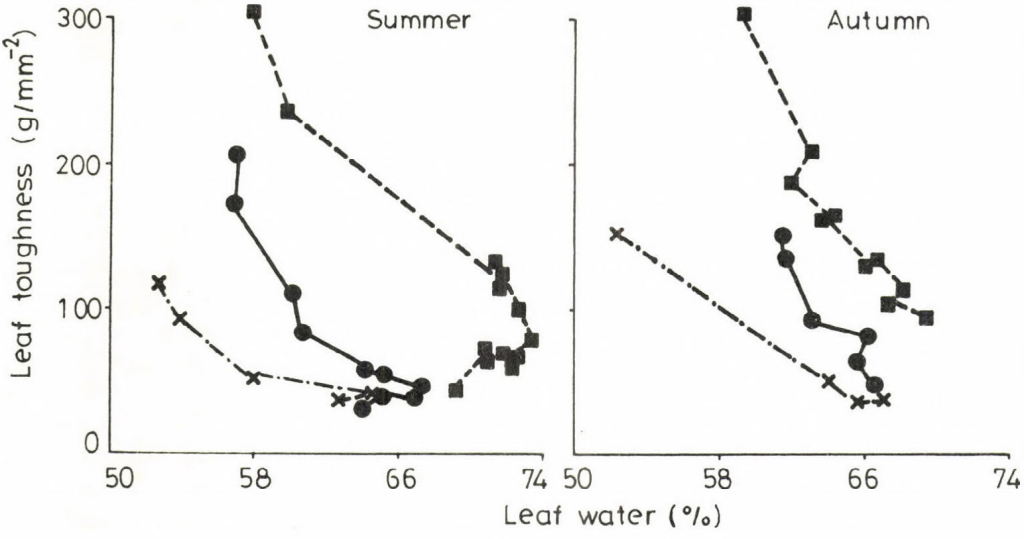

Fig. 1 Relationship between toughness and water content of leaves on stems of three species of eucalypt, measured in summer and autumn. Lines connect the measurements for successive leaves on each stem. For *E. pauciflora* (■) all leaves were sampled, while for *E. bicostata* (●) and *E. viminalis* (×) one leaf of each pair was sampled.

leaf that was measured with the penetrometer was selected for interspecific comparisons of toughness. The leaf toughness measurements determined by these criteria are presented for all species in both seasons in Table 2. *E. pauciflora* had the toughest foliage on both occasions, and on average was between 1.4 and 3.0 times tougher than the other species.

Effect of leaf waxes on beetle adhesion. The mean weight of the *Paropsis* beetles was three times that of the *Chrysophtharta* beetles. On the non-waxy foliage of *E. pauciflora* and *E. aggregata* the *Paropsis* beetles pulled approximately twice as strongly as the *Chrysophtharta* (Table 3).

All species were able to pull much more strongly on the non-waxy foliage than on the waxy foliage (Table 3). The beetles were unable to obtain a grip on the waxy leaves, and their feet repeatedly slipped on the leaf surface. This was followed by considerable grooming to clean fragments of wax from the tarsal pads. *Paropsis* pulled 7.7 times more strongly, and *Chrysophtharta* 7.6 times more strongly on non-waxy than on waxy foliage.

Laboratory rearing. Prepupal weight and survival of *C. m-fuscum* larvae were approximately equal on *E. macarthurii, E. mannifera, E. bridgesiana* and *E. bicostata* (Table 4). Development was significantly faster on the first three species than on *E. bicostata*, even though *E. macarthurii* and *E. mannifera* were not colonized in the field, and *E. bicostata* was a preferred host (Table 1).

C. 'fluoro green' prepupae were heaviest, had the highest survival rate and fastest development on *E. aggregata* (Table 5), the host on which this species occurred in the field (Table 1). No larvae survived to the prepupal stage on *E. pauciflora* or *E. bicostata*, and most died during the first instar. Larvae repeatedly fell off *E. bicostata*, and were replaced each day until they died.

DISCUSSION

The results indicate that differences in host plant utilization patterns of chrysomelids can, in part, be correlated with variations in the physical attributes of eucalypt leaves. This finding does not reduce the probability of plant chemistry being a major factor in determining the host range of eucalypt-feeding chrysomelids.

E. pauciflora had the toughest leaves and the fewest insects. Although this may not be a causal relationship, the observation that adults and larvae of chrysomelids can feed on *E. pauciflora* in the field (Strauss and Morrow, 1988, unpub. obs.), indicates that the foliage is nutritionally adequate and is not toxic, at least in the short term. Therefore the low number of chrysomelids on *E. pauciflora* is presumably the consequence of adult beetles avoiding *E. pauciflora*, or remaining on the foliage for only a short time.

Ohmart et al., (1985) found that leaf toughness was a major cause of mortality of *P. atomaria* larvae reared on *E. blakelyi*, particularly for early instar larvae. The toughness of *E. blakelyi*, at which significant larval mortality occurred was 62 g/m^2. The results from the present study indicate that the toughness of young *E. pauciflora*

Table 4 Prepupal weight, survival and developmental period of *C. m-fuscum* larvae on foliage of eight *Eucalyptus* species in the laboratory (23°C : 15°C, 12hL : 12hD). Each treatment consisted of 2 cohorts of 10 larvae (n = 20).

Foodplant	Prepupal weight X±SD (mg)	Larval survival (%)	Duration of larval development X±SD (days)
E. macarthurii	43.0±6.6[a]	55	15.0±2.5[a]
E. mannifera	39.7±6.5[a]	80	15.9±2.7[a]
E. bridgesiana	38.8±6.1[a]	60	16.5±1.9[a]
E. bicostata	40.6±7.0[a]	70	21.8±2.8[b]
E. viminalis	(49.5±7.6)[1]	10	19.0±1.4[b]
E. melliodora	-	0	-
E. pauciflora	-	0	-
E. aggregata	-	0	-

[a, b] Consecutive pairs of means in each column that are followed by different superscripts are significantly different, t = test, $P<0.05$

[1] The two larvae that survived in this treatment were both females. Since females were heavier than males, this treatment cannot be compared with the others, in which the sexes were approximately equally represented.

foliage is well above this level (90 g/m^2, Table 2). In the laboratory, most larvae of *C 'fluoro green'* and *C. m-fuscum* reared on *E. pauciflora* died during the first instar, the stage when they would be most susceptible to leaf toughness.

Six chrysomelid species were most abundant on *E. viminalis* and *E. macarthurii*, the eucalypts with the softest leaves (Table 2). The oil profiles of these two eucalypts are markedly different (J. Murrell, pers. comm.), so it is likely that their physical suitability for these six chrysomelids is of more consequence than chemical differences. The fact that chrysomelid larvae and adults almost invariably feed only on the soft young foliage of eucalypts also supports the contention that leaf toughness may be an important variable in determining niche breadth.

The waxy bloom on the leaves of *E. bridgesiana* and *E. bicostata* prevented adult chrysomelids from obtaining a grip on the leaf surface (Table 3). Since trees are colonized by flying adults, this phenomenon may confer considerable protection on species with waxy leaves. Additionally, many chrysomelids lay their eggs while clinging to the flat surface of the leaf. Thus good adhesion to the substrate is essential. Larger insects such as Christmas beetles (Scarabaeidae: *Anoplognathus*) are not as constrained by the nature of the leaf surface, since they are large enough to grip the leaf edge, the petiole and/or stem with their long opposable claws while feeding (Carne et al., 1974).

In light of the above result, the field distribution of *C. m-fuscum* was surprising, since it occurred almost exclusively on the waxy-leafed *E. bridgesiana* and *E. bicostata*. Adults of *C. m-fuscum*, were hampered by surface waxes as were the other chrysomelid species (Table 3), indicating that *C. m-fuscum* has no special modifications of the feet for overcoming the slipperiness of the leaf surface. However it seems likely that behavioural and physical adaptations enable *C. m-fuscum* to occupy a

Table 5 Prepupal weight, survival and developmental period of *C. 'fluoro green'* larvae on foliage of eight *Eucalyptus* species in the laboratory (23°C : 15°C, 12hL : 12hD). Each treatment consisted of 3 cohorts of 10 larvae (n = 30).

Foodplant	Prepupal weight X±SD (mg)	Larval survival (%)	Duration of larval development X±SD (days)
E. aggregata	67.3 ± 8.9^{a}	83	15.0 ± 2.0^{a}
E. mannifera	58.9 ± 8.7^{b}	69	18.4 ± 0.9^{b}
E. melliodora	58.9 ± 9.4^{b}	53	17.8 ± 2.0^{b}
E. macarthurii	56.1 ± 7.0^{b}	24	17.2 ± 2.3^{b}
E. bridgesiana	52.3 ± 3.2^{b}	10	25.7 ± 2.1^{c}
E. viminalis	50.2 ± 9.9^{b}	27	21.3 ± 2.9^{d}
E. pauciflora	-	0	-
E. bicostata	-	0	-

^{a,b,c,d} Consecutive pairs of means in each column that are followed by different superscripts are significantly different, t = test, $P<0.05$

niche that is largely unavailable to other chrysomelid species. These modifications are to be discussed in detail elsewhere, but include oviposition behaviour, selection of resting sites, size, colour and microsculpture on first instar larvae.

P. aegrota was also abundant on the waxy-leafed eucalypts, *E. bridgesiana* and *E. bicostata* (Table 1). *P. aegrota* lays its eggs on the edge of the leaf. By straddling the leaf edge during oviposition, *P. aegrota* females may prevent themselves from slipping off the leaf. *P. aegrota* larvae are often seen resting on stems of the plant with their abdomens flexed upwards, behaviour that may reduce the chance of slipping from leaves or becoming contaminated by wax. When *C. 'fluoro-green'* was reared on *E. bridgesiana* and *E. bicostata* in the laboratory, the larvae repeatedly slipped off the foliage, and became entangled in wax. In contrast, *C. m-fuscum* larvae did not slip off the foliage. *P. atomaria* larvae performed extremely poorly on the juvenile foliage of *E. bicostata* (Carne, 1966), perhaps for the same reasons as for *C. 'fluoro green'*.

In spite of the strong preference of *C. m-fuscum* for *E. bridgesiana* and *E. bicostata* in the field, in the laboratory it performed as well on *E. macarthurii* and *E. mannifera* as on the preferred hosts, in terms of larval survival and prepupal weight, and in fact developed faster on one of the non-hosts (Table 4). This indicates that in no-choice experiments a broad range of hosts is acceptable to *C. m-fuscum*. This is not an unusual result. *P. atomaria* and *C. agricola* larvae also performed better in the laboratory on non-preferred than on preferred hosts (Carne, 1966; de Little and Madden, 1975). To speculate on the significance of this is academic, since (i) survival and development rates in the field may differ from those in the laboratory, due to natural enemies and other factors, and (ii) eggs and larvae of *C. m-fuscum* were never found on the non-hosts. This suggests that discrimination between host plants is effected by ovipositing females. In summary, it appears that the strong host preference of *C. m–fuscum* is the result of it having overcome the physical difficulties

associated with colonizing waxy-leafed species, rather than a close physiological relationship between it and its hosts.

In contrast, the very strong host preference of *C. 'fluoro green'* for *E. aggregata* was not correlated with physical attributes of the foliage, and may well be a consequence of physiological adaptations. In the field, *E. aggregata* was largely avoided by the other chrysomelids, but 91% of *C. 'fluoro green'* were found on this species. In the laboratory, larval performance of *C. 'fluoro green'* was best on the preferred host, *E. aggregata*, for the three parameters measured (Table 5), while survival of *C. m-fuscum* was zero on *E. aggregata* (Table 4). It seems probable that *E. aggregata* is protected against most chrysomelids by its leaf chemistry, but not against *C. 'fluoro green'*, which may be able to tolerate or detoxify the offending chemicals.

Determining the degree of host specificity of eucalypt-feeding insects has generated considerable interest at a theoretical level, particularly in relation to its possible role in maintaining the stable associations that exist between co-dominant eucalypt species (Burdon and Chilvers, 1974; Morrow, 1977). However there are also practical benefits to be derived from determining the host preferences of eucalypt insects and in understanding the processes involved. Increasingly, eucalypts are being replanted on farms and degraded land in Australia, and grown in plantations for pulp and timber production in Australia and overseas. Even-aged monospecific stands create a greatly increased risk of substantial insect damage. Chrysomelids have already caused problems in plantations in Tasmania and New Zealand (Greaves, 1966; Styles, 1970). While damage has been severe in both localities, it has been observed that the glaucous juvenile foliage of *E. nitens* and *E. globulus* has been virtually untouched by chrysomelids (Edwards, 1982; Ohmart pers. comm.). If differences such as this can be interpreted in terms of the physical and chemical properties of eucalypts that affect the host range of insects attacking the trees, then informed management decisions can be made regarding the species to be planted and the selection of resistant varieties.

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REFERENCES

Burdon, J. J. and G. A. Chilvers (1974): Fungal and insect parasites contributing to niche differentiation in mixed species stands of eucalypt saplings. *Aust. J. Bot. 22*, 103-114.

Carne, P. B. (1966): Ecological characteristics of the eucalypt-defoliating chrysomelid *Paropsis atomaria* Ol. *Aust. J. Zool. 14*, 647-672.

Carne, P. B., T. G. Greaves and R. S. McInnes (1974): Insect damage to plantation-grown eucalypts in north coastal New South Wales, with particular reference to christmas beetles (Coleoptera: Scarabaeidae). *J. Aust. Entomol. Soc. 13*, 189-206.

de Little, D. W. and J. L. Madden (1975): Host preference in the Tasmanian eucalypt defoliating Paropsini (Coleoptera: Chrysomelidae) with particular reference to *Chrysophtharta bimaculata* (Oliver) and *C. agricola* (Chapuis). *J. Aust. Entomol. Soc. 14*, 387-394.

Edwards, P. B. (1982): Do waxes on juvenile *Eucalyptus* leaves provide protection from grazing insects? *Aust. J. Ecol. 7*, 347-352.

Feeny, P. (1970): Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology 51*, 565-581.

Fox, L. R. and P. A. Morrow (1981) Specialization: species property or local phenomenon? *Science 211*, 887-893.

Greaves, R. (1966): Insect defoliation of eucalypt regrowth in the Florentine Valley, Tasmania. *Appita 19*, 119-126.

Macauley, B. J. and L. R. Fox (1980): Variation in total phenols and condensed tannins in *Eucalyptus*, leaf phenology and insect grazing. *Aust. J. Ecol. 5*, 31-35.

Morrow, P. A. (1977): Host specificity of insects in a community of three co-dominant *Eucalyptus* species. *Aust. J. Ecol. 2*, 89-106.

Ohmart, C. P., L. G. Stewart and J. R. Thomas (1985): Effects of food quality, particularly nitrogen concentrations, of *Eucalyptus blakelyi* foliage on the growth of *Paropsis atomaria* larvae (Coleoptera: Chrysomelidae). *Oecologia 65*, 543-549.

Pryor, L. D. (1959): Species distribution and association in *Eucalyptus*. In: A. Keast, R. L. Crocker and C. S. Christian (eds): *Biogeography and Ecology in Australia.* Junk, The Hague. 461-471.

Schillinger, J. A. and R. L. Gallun (1968): Leaf pubescence of wheat as a deterrent to the cereal leaf beetle, *Oulema melanopus*. *Ann. Entomol. Soc. Am. 61*, 900-903.

Simmons, D. and R. F. Parsons (1987): Seasonal variation in the volatile leaf oils of two *Eucalyptus* species. *Biochem. Syst. Ecol. 15*, 209-215.

Stork, N. E. (1980a): Role of waxblooms in preventing attachment to brassicas by the mustard beetle, *Phaedon cochleariae. Entomol. exp. appl. 28*, 100-107.

Stork, N. E. (1980b): Experimental analysis of adhesion of *Chrysolina polita* (Chrysomelidae: Coleoptera) on a variety of surfaces. *J. exp. Biol. 88,* 91-107.

Strauss, S. Y. (1988): The Chrysomelidae: a useful group for investigating herbivore-herbivore interactions. In: P. Jolivet, E. Petitpierre and T. H. Hsiao (eds), *Biology of Chrysomelidae*. Kluwer Academic Publishers, Dordrecht. 91-105.

Strauss, S. Y. and P. A. Morrow (1988): Movement patterns of an Australian chrysomelid beetle in a stand of two *Eucalyptus* host species. *Oecologia 77*, 231-237.

Styles, J. H. (1970): Notes on the biology of *Paropsis charybdis* Stal. (Coleoptera: Chrysomelidae). *N. Z. Entomol. 4*, 103-111.

Tanton, M. T. (1962): The effect of leaf "toughness" on the feeding of larvae of the mustard beetle *Phaedon cochleariae* Fab. *Entomol. exp. appl. 5*, 74-78.

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CONTROVERSIAL COMPONENTS OF PLANT APPARENCY IN *ALLIARIA PETIOLATA* CAVARA & GRANDE (CRUCIFERAE)

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ABSTRACT

The importance of components of apparency for the two growth forms (rosette and stem) of *A. petiolata* is evaluated, and the term "intraspecific (developmental)" apparency is proposed.

Key words: rosette-plant, stem-plant, biennial cycle, *Pieris napi, P. brassicae, Anthocharis cardamines, Ceutorhynchus* spp., architectural complexity, herbivore load, plant distribution, intraspecific (developmental) apparency, herbivorous guilds, herbivore impact.

INTRODUCTION

The theory of plant apparency has had a stimulatory impact on the development of plant antiherbivore strategies, since the term was introduced by Feeny (1975, 1976), and by Rhoades and Cates (1976). The theory was further elaborated (Feeny, 1983), but at the same time also criticized (e.g. Fox, 1981; Crawley, 1983) both on theoretical and experimental grounds. As the theory is generally well-known and is also reviewed (Feeny, this volume), here only certain aspects are discussed.

In short, the term expresses the probability by which a plant species or individual is thought to be discovered by herbivores, which in turn is connected with a set of complex characters. In order "to go unnoticed", plant species may employ different strategies in space and time.

"Apparency", among others, depends on architectural complexity (Lawton and Schröder, 1977), leaf/flower colour, chemical constituents, neighbouring species' similar characters within the plant community, rate/speed of growth, "timing" of vital processes, such as flowering, etc. To treat such an all-inclusive term that combines each important attributes of a plant's life is the more difficult, as we still know very little about the stimuli guiding herbivorous insects to their host-plants.

From community ecological point of view, diversity of a phytophagous insect guild on any given plant species is supposed to be proportional to the available niches, what in turn to a large extent depends on plant structural characters, i.e. architectural complexity (Moran, 1980; Lawton, 1983). Furthermore, depending on the insect's body size the fractal dimensionality of a plant species may vary considerably (Morse

Table 1 Components of apparency contributing to differences in strategies followed by the two growth-forms of *Alliaria petiolata*.

Rosette-form (unapparent)	Stem-form (apparent)
Late, but durable (predictable)	Early, but ephemeral
Vegetative growth only	Vegetative and reproductive growth
Low apparency, low architectural complexity	High apparency, high architectural complexity
Slightly lower phytophage diversity, and lack of pollinators, nectarium-visitors, etc.	Slightly higher phytophage diversity, presence of flower and silique consumers
Low damage compensation ability	High damage compensation
Clumped occurence	Random distribution
Defense against generalists[1]	Less defended against generalists

[1] Larvae of an extreme polyphagous noctuid, *Mamestra brassicae*, in preliminary experiments, consumed ca. three times more of the stem-plant leaves in comparison with those of rosette-plants

et al., 1985; Lawton, 1986). Apparency and its components, most likely, are temporary attributes of a plant species because of changes in the plant's chemistry, size, architecture, etc. as it grows. Therefore, only an average apparency of a species is probable [see another definition of average apparency with Fox (1981)].

The classical objects for apparency studies have been the cruciferous species (Feeny, 1977; Courtney, 1985; Chew, 1988, and others).

In order to study quantitative features of plant-insect interaction from the aspect of apparency, I have chosen a member of this family, the garlic mustard, *Alliaria petiolata*.

MATERIALS AND METHODS

The study area was a ca. 2 x 1 km size forest edge (main species: *Quercus cerris, Carpinus betulus, Sorbus* spp., *Prunus* spp., *Cornus mas*, *Crataegus* spp., all on limestone), located near Budapest at an elevation of ca. 300 m above sea level.

Populations of the two forms of *A. petiolata* were regularly censused, observed and sampled for herbivorous insects throughout several growing seasons.

RESULTS AND DISCUSSION

A. petiolata, a biennial nitrophilous weed, is frequent at humid forest edges. Its two forms: rosette- and stem-form strikingly differ in architectural complexity, but in other characters, too (Fig. 1). The seeds germinate in mid-April, the young plant sprouts 1-4 leaves (rosette-form) and develops a strong root-system of which the probable function is storage. The rosette-form is present throughout the year, growing even during mild winters. The stem-form, starts to produce stems and leaves as early as in February, but usually in March. It grows fast, flowering is terminated by the end of May and siliques are ripe at the end of June. Seeds are spread continuously

during summer. Table 1 lists qualitative differences between the two forms from the point of view of plant apparency theory. The seasonal durabilities of the two forms differ, and this explains differential predictability and availability. While architectural complexity and visibility of stem-form is much higher, the rosette-form has a longer seasonal durability, therefore, is more predictable for herbivores. This trait may counterbalance the supposed unapparent character of the latter.

Quantitative differences are summarized in Table 2. While the rosette-form shows a purely vegetative growth and produces a large root of which wet-mass ratio to above-ground plant parts is slowly increasing, the presence of generative organs on the stem-form dramatically changes the architectural complexity of the latter. Also, microsite differences (e.g. edaphic conditions, duration of exposure to sun) may strongly alter size, number of leaves, etc. of plants. For example, at a sunny place the sum of leaf surface/stem-plant (average of 11 plants) was 48 ± 17 cm^2, while the

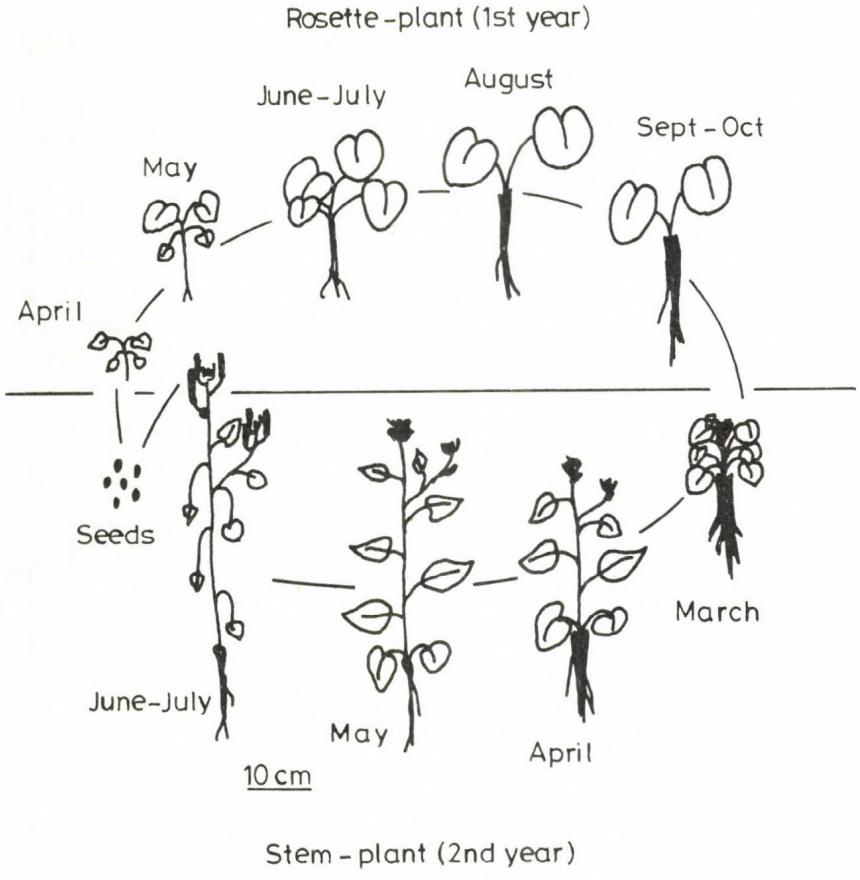

Fig. 1 Schematic drawing of the developmental cycle of *A. petiolata*.

Table 2 Generalized quantitative differences, as elements of architectural complexity and apparency between rosette- and stem-form of *A. petiolata*.

Characters	Rosette-form	Stem-form
Plant height (cm)	3-10	20-100
No. of stalks	none	1-5
No. of leaves	1-4	6-20
Size of leaves (cm^2)	1-80	1-100
No. of ramifications	none	1-5
Above-ground: root wet-mass ratio (root = 1)	1:3.5[1]	max. 1:10
Wet mass (g)	0.5-1	>5-6
No. of inflorescence/plant	none	3-8
No. of siliques	none	1-30

[1] It drops from 1:40 (germination) to 1:3.5 by July.

same character at a shady surrounding was 390 ± 115 cm^2. Rosette-plants have a leaf surface/plant of 63 ± 21 cm^2 (mean ± S.D.).

Although the two growth-forms differ considerably in the components of apparency the species compositions of herbivorous guilds turned out to be very similar on both (Table 3), only *Anthocharis cardamines* (Lep., Pieridae) and the aphids were restricted to one of them. This means that the apparency of the two growth-forms must be different for the two groups, but may be similar for the rest. Curculionid species most probably react to chemical plant characters. Living on and pupating under a rosette stand increases the probability of finding stem-plants at the same site next year. Herbivore populations, to some degree, do segregate in space and time in exploiting the two forms. Population levels of all species are generally low (Table 4).

Table 3 Herbivores on the two growth forms of *A. petiolata*.

Species/group	Rosette-plant	Stem-plant
Halticinae (*Phyllotreta*, *Aphtona*, and *Longitarsus* spp.)	+[1]	+
Curculionidae (at least 5 *Ceutorhynchus* species)	+	+[2]
Lepidoptera	+	+
Pieris napi		
Anthocharis cardamines	–	+
Evergestis forficalis	+	(+)
Tenthredinidae	+	+
Aphids	+[3]	–
Slugs, snails	+	+

[1] + = present, - = absent.
[2] Some might have specialized to certain regions of the plant.
[3] Seasonal, stem plant is not available when the aphids appear (from September on).

Table 4 Seasonal herbivore load on rosette- and stem-forms of *A. petiolata*. (50-100 plants examined per sample.) 1985.

Census date		"Galls"		Halticinae		Curculionids		Pierid egg/larva		Others	
		R[1]	S[2]	R	S	R	S	R	S	R	S
April	3	-[3]	0	-	13	-	0	-	0	-	0
	10	-	11	-	37	-	0	-	0	-	0
	17	-	60	-	11	-	1	-	0	-	0
	24	16	89	0	17	0	5	0	0	0	3
May	7	18	140	2	6	0	0	0	0	0	1
	15	42	124	0	2	0	1	0	2	0	2
	22	97	161	0	0	0	2	0	1	3	0
	29	71	87	0	0	0	0	0	2	0	1
June	5	92	202	0	0	1	10	0	1	1	3
	12	99	131	0	0	2	4	1	2	0	6
	19	104	91	0	0	0	8	0	0	0	1
	26	87	105	0	4	0	8	0	2	0	2
July	3	123	59	0	2	7	3	0	0	3	3
	10	83	43	2	6	5	13	0	0	0	3
	17	82	65	1	12	9	29	1	1	0	3
	24	56	46	2	12	16	13	0	1	0	5
	31	48	-[4]	4	-	12	-	2	-	0	-
August	9	33	-	1	-	1	-	4	-	1	-

[1] R=Rosette form.
[2] S=Stem-form.
[3] -=No rosette-form was available yet that time.
[4] -=No stem-form was available anymore from that time

Many attributes of crucifer life-history are assumed to serve as components of successful escape from herbivores (Chew, 1988). However, there are others that can increase the probability of host finding. Such a factor is, for instance, the spatial and timely coexistence of the two growth- (rosette- and stem-) forms. Still another factor is the patchy distribution of plants. Cain (1985) modelled plant density and herbivore damage relations and found that at high plant densities herbivore damage was low, while the opposite was expected at low plant densities. Cain's model was strengthened by the results of Auerbach and Shmida (1987) and data collected in a review by Antonovics and Levin (1980). My observations were also in agreement with the model in 1985: at 7 clumped plant stands the number of stem-plants was $29 \pm 12/m^2$ (mean±S.D.), and the number of gall-like tissue-proliferations caused by *Ceutorhynchus* species was approx. 1/plant, while at places where the density of plants dropped to $1.2/m^2$, the number of "galls" was 4.2/plant. The above data do not support the resource concentration hypothesis (Root, 1973).

Table 5 Quadrat-sized yearly and seasonal population fluctuations of the two forms of *A. petiolata* (means).

Year/month		Grid I[1]		Grid II[2]	
		Rosette-	Stem Plants/1 m^2	Rosette-	Stem-
1986	April	12.2	0	5.4	0
	May	12.3	0	6.4	0
1987	April	3	0.3	1.5	0.04
	May	4.7	0.1	4.8	0.04
	June	4.5	0.1	5.1	-[3]
	July	2.7	-[3]	2.8	
	Sept.	1.5		2.2	
1988	April	1.3	0.1	0.6	0.6
	May	1.1	0	0.5	0.5
	June	0.7	0	0.25	0.37
	July	0.05	0	0.1	0.1
	August	-	-	0.04	-[3]

[1] Grid I was a permanent census site. Size was 10 m^2, 5 quadrats had been seeded with 100 seeds each in 1984.
[2] Grid II was another permanent census site. Size was 24 m^2, no seeding occurred. They were 100 m apart, and had similar surrounding vegetations.
[3] The stem-plants died by this time.

The above are partly the consequences of an alternating distribution pattern existing between the two forms. Stem-plants disperse the seeds in the immediate vicinity, therefore, the occurrence of a clumped rosette population at the same place next year is highly probable. Mortality factors decrease the number of rosette plants so that in the second year stem-plants will tend to show a random distribution. Yearly and seasonal large-amplitude (2 orders of magnitude) plant population fluctuations (Table 5) do serve as escape mechanisms. However, such fluctuations are frequently the consequence of abiotic factors (e.g. water shortage, low availability of microsites for germination, etc.), and additional herbivore impacts will only accelerate local extinction. Such is the case with rosette-plants, where the impact of summer water deficiency, further increased by the intensive curculionid feeding (peeling), eventually leads to the complete loss of photosynthetic surface and to mortality. While some seasonal fluctuations are compensated by the temporary coincidence of the two growth-forms, or compensatory regrowth, the total absence of one form locally in any one year or the senescing and dying off of the stem-form, may result in a disproportionate impact by herbivores, such as some curculionid (*Ceutorhynchus*) species living on both forms.

Visual components of apparency affect herbivore populations differentially. The orange tip, *A. cardamines* lays its eggs only during flowering of the plant, and avoids it outside this time period. A dense vegetation around a rosette-plant may hide it from phytophagous insects searching visually. Chew (1975, 1977) pointed out that

ovipositing butterflies confused plants either on the chemical basis or by the shape and size. Likewise, I observed that alighting response by *Pieris napi* (Lep., Pieridae) females on other plant species morphologically only rarely resembling the rosette-form of *A. petiolata*, was frequent in an area where they commonly occurred with the host-plant. Also in an open field experiment I noticed that a *P. rapae* (Lep., Pieridae) female alighted and oviposited on a *Galinsoga* species (family Compositae) in the abundant presence of the host-plant, *A. petiolata*.

Summarizing, the following may be outlined: 1) The herbivorous guilds of the two forms are *almost identical* which is the consequence of a) the similar host finding mechanisms employed in some insect groups (Halticinae, Curculionidae, Tenthredinidae) and by snails and slugs, and, b) the simultaneous presence of the two forms. 2) The *differences* existing between the rosette- and stem-forms means a difference also in apparency, e.g. for the possibly visually searching *A. cardamines*, while no such distinction is made by other two pierid butterflies, i.e. the apparency of the two plant forms seems to be similar for them. 3) Distribution patterns of plant forms regularly change and this has a bearing on the size and impact of the herbivorous guilds. 4) The composition of the herbivorous guilds on both plant forms are not influenced by the available niches. While the "leaf-chewing guild" comprises 7 members (species), there are no root consuming, seed predator, etc. guilds.

The two growth-forms of *Alliaria* possess two kinds of apparency that may result in different survival strategies, and the species as such, can be characterized by a mixture of these. During ontogenetic development the quality of apparency changes. Therefore, it may seem justifiable to make a distinction between inter- and intraspecific apparencies. Intraspecific (developmental) apparency comprises traits that explain plant strategies during ontogeny.

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REFERENCES

Antonovics, J. and D. A. Levin (1980): The ecological and genetic consequences of density-dependent regulation in plants. *Ann. Rev. Ecol. Syst. 11*, 411-452.

Auerbach, M. and A. Shmida (1987): Patch formation among Israeli crucifers: how do they get away with it? *Ann. Miss. Bot. Gard. 74*, 583-594.

Cain, M. L. (1985): Random search by herbivorous insects: a simulation model. *Ecology 66*, 876-888.

Chew, F. S. (1975): Coevolution of pierid butterflies and their cruciferous foodplants. I. The relative quality of available resources. *Oecologia 20*, 117-127.

Chew, F. S. (1977): Coevolution of pierid butterflies and their cruciferous foodplants. II. The distribution of eggs on foodplants. *Evolution 31*, 568-579.

Chew, F. S. (1988): Searching for defensive chemistry in the Cruciferae, or, do glucosinolates always control potential interactions of Cruciferae with their potential herbivores and symbionts? No! In: K. S. Spencer, (ed), *Chemical Mediation of Coevolution.* Acad. Press, San Diego. 81-112.

Courtney, S. P. (1985): Apparency in coevolving relationships. *Oikos 44*, 91-98.

Crawley, M. J. (1983): *Herbivory. The Dynamics of Animal-Plant Interactions*. Blackwell Sci. Publ., Oxford.

Feeny, P. P. (1975): Biochemical coevolution between plants and their insect herbivores. In: L. E. Gilbert, and P. H. Raven (eds), *Coevolution of Animals and Plants.* University Texas Press, Austin. 3-19.

Feeny, P. P. (1976): Plant apparency and chemical defense. In: Wallace, J. W. and R. L. Mansell (eds), *Biochemical Interaction between Plants and Insects*. Plenum Press, New York. 1-40.

Feeny, P. P. (1977): Defensive ecology of Cruciferae. *Ann. Missouri Bot. Gard. 64*, 221-234.

Feeny, P. P. (1983): Coevolution of plants and insects. In: Whitehead, D. L. and W. S. Bowers (eds), *Natural Products for Innovative Pest Management*. Pergamon Press, Oxford. 167-184.

Fox, L. R. (1981): Defense and dynamics in plant-herbivore systems. *Amer. Zool. 21*, 853-864.

Lawton, J. H. (1983): Plant architecture and the diversity of phytophagous insects. *Ann. Rev. Entomol. 28*, 23-39.

Lawton, J. H. (1986): Surface availability and insect community structure: the effects of architecture and fractal dimension of plants. In: B. Juniper and R. Southwood (eds) *Insects and the Plant Surface.* Edward Arnold, London. 317-332.

Lawton, J. H. and D. Schröder (1977): Effects of plant type, size of geographical range and taxonomic isolation on number of insect species associated with British plants. *Nature 265*, 137-140.

Moran, V. C. (1980): Interactions between phytophagous insects and their *Opuntia* hosts. *Ecol. Entomol. 5*, 153-164.

Morse, D. R., J. H. Lawton, M. M. Dobson, and M. H. Williamson (1985): Fractal dimension of vegetation and the distribution of arthropod body lengths. *Nature 314*, 731-732.

Rhoades, D. F. and R. G. Cates (1976): Toward a general theory of plant antiherbivore chemistry. In: J. W. Wallace, and R. L. Mansell (eds), *Biochemical Interaction between Plants and Insects*. Plenum Press, New York. 168-213.

Root, R. B. (1973): Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecol. Monogr. 43*, 95-124.

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GENETIC VARIATION IN LIFE CYCLE AND HOST PLANT RELATIONS IN *PEMPHIGUS BETAE*

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ABSTRACT

A population of the aphid, *Pemphigus betae*, in Utah includes lineages that remain year-round on roots of herbaceous hosts and also lineages that host alternate between these hosts and *Populus angustifolia*. Only slight differences in thresholds for producing different morphs are needed to cause a switch from alternating to non-alternating life cycles; thus, it is hypothesized that small genetic changes may result in dramatic changes in host relations. Aphids are parthenogenetic and form natural clones. Thus, the total genetic component of life cycle variation may be isolated from environmental components by rearing sublines of clones separately and using ANOVA to isolate variation due to genotype from variation due to environmental (subline) effects. Preliminary results for eight clones show that all clones could produce the two types of overwintering morph corresponding to both types of life cycle. However, clones differed significantly in their relative levels of production of the two types. This genetic variation in life cycle characteristics may be subject to local selection due to differences in suitability of *Populus* hosts at different sites.

Key words: aphid, genetic variation, heteroecy, host alternation, life cycle variation, local adaptation, *Pemphigus*.

INTRODUCTION

In many insects, patterns of host plant utilization are greatly influenced by the nature and timing of life cycle phenomena. Relatively small changes in the environmental cues and physiological thresholds that regulate life cycles can result in dramatic shifts in patterns of host use.

Host-alternating aphids provide one of the most dramatic examples of how small genetic changes can result in large changes in life cycle and host relations. In these aphids, two different sets of hosts are utilized at different times of the season (Hille Ris Lambers, 1980; Dixon, 1985). While the origins of host alternation may have been infrequent and largely ancient (Moran, 1989), the secondary reduction of alternating life cycles to non-alternating ones occurs more frequently through the loss of the morphs corresponding to one of the two alternative sets of host plants (Hille Ris Lambers, 1950; Blackman and Eastop, 1984; Moran, 1988). In host-alternating aphids, a change in relative frequencies of alternative morphs in autumn can result in decreased use or elimination of one of the alternate host-plants, accompanied by

corresponding increased use of the other set of hosts. If there is genetically based variation in tendency to produce these alternative morphs, characteristics of locally available hosts may act as selective factors influencing the frequency of the host alternating life cycle and the relative importance of the alternative hosts in the life cycles.

ORGANISM AND METHODS

Pemphigus betae Doane (Homoptera: Aphididae: Pemphiginae) shows a variable complex life cycle, in which it may alternate between narrowleaf cottonwood (*Populus angustifolia*) and roots of *Rumex, Chenopodium* and *Polygonum*, or it may remain throughout the year on the latter, "secondary" host plant (Moran and Whitham, 1988). In autumn, *P. betae* clones can produce either root-overwintering females that remain parthenogenetic on roots of secondary hosts and/or winged sexuparae that fly to cottonwood and produce the sexual generation (Moran and Whitham, 1988). A series of studies have been directed at determining the extent of genetic and environmental variation underlying this life cycle variation and the role of host plant features as selective agents affecting life cycle expression in *P. betae*. Earlier work involving common garden and field transfer experiments established that there are both genetic and environmental components to the life cycle variation that exist both within and between sites in the Weber Canyon, Utah, USA (Moran and Whitham, 1988).

Because *P. betae* can be reared indefinitely in a clonal state, the genetic component of life cycle variation can be estimated by rearing individual clones in multiple sublines, characterizing their life cycle characteristics, and using analysis of variance to determine the extent of clone effects on the characters of interest (Lynch, 1984). In recent work on this system, clonal sublines reared in the laboratory have been used to determine the extent of genetically based variation in production of these alternate morphs. Clones were established with single females both from galls on cottonwood and from colonies which had overwintered on roots. They were grown under constant conditions on *Rumex patientia* roots in sealed containers in growth chambers 15L/9D and constant 20°C. Under these conditions, sexupara production begins approximately 14 weeks after clones are initiated from field collections. Clonal sublines were monitored for six weeks during the period of sexupara production. During this interval, all sexuparae were removed and counted.

RESULTS AND DISCUSSION

All clones tested were able to produce both root-overwintering (non-alternating) morphs and also winged sexuparae which fly to alternate host plants. Thus, all clones have the capacity to host-alternate or not during a particular season. However, preliminary results from a set of eight clones indicate highly significant variation

Table 1 Production of sexuparae by eight clones of *Pemphigus betae*. Clones were initiated by single females collected from galls on *Populus angustifolia* or from colonies overwintering on roots of *Rumex patientia*. Each clonal mean is for several sublines grown under identical conditions. ANOVA: $F(7,18)=6.08$, $p<0.002$

Clone origin	# sublines	mean of $\log_{10}$ (# sexuparae produced/subline)	standard deviation
root	4	0.46	0.64
root	6	0.83	0.71
root	3	0.88	0.28
root	2	0.75	0.15
gall	2	1.48	0.30
gall	3	1.10	0.11
gall	2	0.30	0.3
gall	3	0.99	0.49

among clones in level of sexupara production under identical conditions (Table 1). Thus, certain genotypes remain on root host plants longer than do others. This genetically based variation in life cycle suggests that selection acts to to adjust the frequency of host alternation within this population.

Local selection on life cycle due to variation in host plant quality appears to occur in the Weber Canyon. The cottonwood hosts of *P. betae* in the Weber Canyon include both pure *P. angustifolia* and also backcrossed hybrids between *P. angustifolia* and *P. fremontii*. Whitham (1989) has found that the most favorable trees for gall formation are trees with hybrid backgrounds. These trees occur primarily in one zone of the canyon, where the distributions of the parental tree species overlap. Collections from this zone produce more migrants that go to cottonwoods than do collections from upper elevations where only the more resistant, pure *P. angustifolia* occur (Moran and Whitham, 1988). Thus, the distribution of these most favorable cottonwood hosts may have influenced the composition of local *P. betae* populations.

REFERENCES

Blackman, R. L. and V. F. Eastop (1984): *Aphids on the World's Crops*. John Wiley and Sons, New York.

Dixon, A. F. G. (1985): *Aphid Ecology*. Chapman and Hall, New York.

Hille Ris Lambers, D. (1950): Host plants and aphids classification. *Proc. Int. Congr. Entomol. (Stockholm) 8,* 141-144.

Hille Ris Lambers, D. (1980): Aphids as botanists? *Symp. Bot. Uppsal. 22,* 114-119.

Lynch, M. (1984): The limits to life history evolution in *Daphnia*. *Evolution 38,* 465-482.

Moran, N. A. (1988): The evolution of host alternation in aphids: Evidence that specialization is a dead end. *Am. Nat. 132,* 681-706

Moran, N. A. (1989): A 48-million year old aphid-host plant association and complex life cycle: biogeographic evidence. *Science 245*, 173-175.

Moran, N. A. and T. G. Whitham (1988): Evolutionary reduction of complex life cycles: loss of host-alternation in *Pemphigus* (Homoptera: Aphididae). *Evolution 42,* 717-728.

Whitham, T. G. (1989): Plant hybrid zones as sinks for pests. *Science 244,* 1490-1493.

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NOCTURNAL REST AND DISPLACEMENT PATTERNS OF *BRUCHUS AFFINIS* (COL., BRUCHIDAE) ON SPECIFIC *LATHYRUS* SSP. (LEGUMINOSAE) POPULATIONS

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ABSTRACT

Exact observations of the behaviour of *Bruchus affinis* Frölich (Col., Bruchidae) in *Lathyrus* fields, confirmed by diurnal and nocturnal experiments, showed that the adults of the bruchid systematically spent the night in the flowers of *L. sylvestris* and *L. pratensis* L. (Leguminosae). In daytime the movements of the adults within the same *Lathyrus* population (studied by capture, marking and recapture method) was relatively limited in space (high percentage of adults recaptured in the area of their release) and was more important in *L. sylvestris* than in *L. pratensis*. *B. affinis* adults use *L. pratensis* for pollen feeding only and *L. sylvestris* for pollen feeding and laying eggs on pods. The possibility of a territorialism playing a role in the pollen dispersion and specifically influenced by the host-plant flowers is discussed.

Key words: *Bruchus affinis*, *Lathyrus*, behaviour, displacement, pollination.

INTRODUCTION

Regarding the biology of bruchids and their behaviour, very little is known on their night and day activities (Southgate, 1979). Among monovoltine species, data are available on the colonization of the host population (Korab, 1927) and the distribution according to the hibernation sites (Wakeland, 1934), but nothing is known on the displacement patterns in relation to the complex relations between the bruchid adults and the specific plant populations.

Bruchus affinis Frölich is a monovoltine bruchid. In Southwest France (Bearn), the adults develop on both *Lathyrus latifolius* L. and *L. sylvestris* L. which are semi-herbaceous vines flowering and producting pods from July to September. In most of the locations studied, *L. latifolius* or *L. sylvestris* are associated with *Lathyrus pratensis* which flowers earlier.

The adults emerge from the *Lathyrus* seeds at the beginning of fall and hibernate throughout the winter. The colonization of *Lathyrus* populations takes place in the middle of May and the adults are first seen on *L. pratensis*. On this species, the beetles are found feeding on pollen but they do not terminate their reproductive diapause. Sexual activities and vitellogenesis start after contact with *L. latifolius* or

Table 1 Number of *B. affinis* caught at different times of the night of June 26-June 27 (9 pm local time = 7 pm GMT)

lot N°	time of exposition from ...	to	number of inflorescences examined	number of bags used	number of adults caught
1	9 pm	5 am	82	40	20
2	9.30	5	83	40	8
3	10.30	5	82	40	0
4	11.20	5	83	40	0

L. sylvestris and the females lay eggs on the green pods of the 2 species (Bashar et al., 1985).

The study of the colonization movements and the displacements of the adult from *L. pratensis* to *L. sylvestris*, or within the same *Lathyrus* population led to interesting observations on the behaviour of the adults and to the hypothesis of territorialism which is the object of the present contribution.

RESULTS

(I) Daytime activity: are the adults attracted to the flower or to the pods?

In the "St. Suzanne" location in a *L. sylvestris* population, 2 isolated tillers, and 5 inflorescencens for each were selected for observations. Two observers looked at the movements of *B. affinis* adults to the flowers and to the pods, from 8.30 am to 8.30 pm. During observations the following were recorded:

(1) The adults were found inside the flowers in the early morning. They leave the flowers at sunrise and start visiting activities when the air temperatures exceeds 20°C. At sunset, when the air temperature is below 20°C, they were found again reaching the flowers and resting in them.

(2) The adults seem to be attracted mainly to the flowers (inflorescences,) into which they penetrate immediately, for pollen consumption (Bashar et al., 1987). The pods are suspected not to attract the bruchids. The adults seem to encounter them when walking at random along the leaves and stems of the *Lathyrus* plants.

II) Nocturnal activity: do the adults spend the night in the flowers?

In the location of "Cadillon" a total of 330 inflorescencens were selected. They were grouped into 4 lots and were hidden into muslin bags or exposed to the visiting bruchids at different time of sunset and the night (Table 1). Before the flowers were exposed, the bruchids found on or in them were eliminated. After 5 hours of exposion, all the inflorescences were closed within the respective bags and the presence of bruchids was checked the next day.

The examination of the flowers revealed that the inflorescences exposed during sunset have given shelter to adults. No adults were found in the flowers exposed after sunset. This confirms that the *B. affinis* adults spend the night inside the flowers, they get into them at sunset, and they do not move during night.

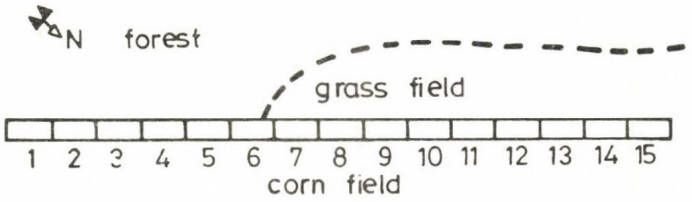

Fig. 1. Experimental plots at St. Suzanne and the surrounding vegetation.

The two previous experiments lead to 2 hypotheses: (1) Flowers give shelter for adults during the night and this could be the origin of a territorialism restricting the area of pollen transportation. (2) Flowers are the exclusive organs capable of attracting the bruchids to the host plant. Therefore, the distribution of adults and eggs in the population is strictly dependant on the distribution of flowers.

To answer these questions, the following experiments were conducted.

(III) Displacement patterns: do *B. affinis* adults move?

The experimental field at "St. Suzanne" is a plain (50 m altitude). *L. sylvestris* mixed with *L. pratensis* were distributed along a line between the edge of a forest and a corn field (Fig. 1). This "population" is not isolated from others in the vicinity. The whole area, estimated 300 m long and 4-5 m wide is divided into 15 plots (20 m x 4 m). From plot 1 to 6, the population is countinously attached to the edge of the forest and from 7 to 15, it is separated from the forest by a grass field (Fig. 1). The study was conducted on the same plots both on *L. sylvestris* and *L. pratensis*.

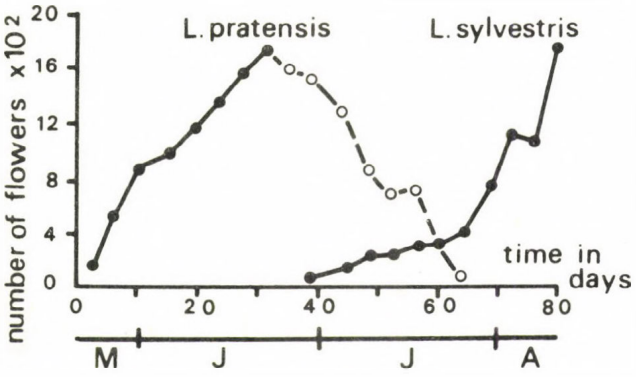

Fig. 2. Abundance of *L. pratensis* and *L. sylvestris* flowers from May 20 to August 13 in the field of St. Suzanne.

From the beginning of May to the end of August, each plot was visited on 3 to 4 days intervals to count the inflorescencens and to perform a "capture-recapture" experiment. The adults were collected in or on the flowers of the different plots from 10 am (plot 1) to about 7 pm (plot 15) on the same day. They were brought into the laboratory and marked according to the plot and flowers of origin. Then they were released in the same plot. The same operation was performed 3 to 4 days later. The data obtained were: 1) the number of inflorescencens, 2) the number of adults captured, 3) the number of adults captured in the same or in a different plot.

L. pratensis flowers are present from the middle of May to the end of June to mid-September (Fig. 2). The displacement of *B. affinis* adults on *L. pratensis* were studied from May 25 to July 2, and from July 6 to August 13 for *B. affinis* on *L. sylvestris*.

The abundance of the beetles on the flowers of both species fluctuated according to the number of the inflorescencens recorded in each plot (Fig. 3). The same observation can be made for the recaptured bruchids: For absolute numbers, the more abundant were the flowers, the more numerous the captured and recaptured beetles were.

The percentage of adults recaptured at 3/4 days intervals was different with the two species: (a) for the number of adults recaptured in the plot of release compared to the number of adults marked and released, we got from 10.8 to 55.4 (June 3) for *L. pratensis* and from 6.8 to 16.5 (August 9) for *L. sylvestris* (Fig. 4); (b) If we compared the number of adults recaptured in the plot of release to the total number of adults recaptured we got higher values: 79.5% on June 3 in *L. pratensis*, and 51.5%

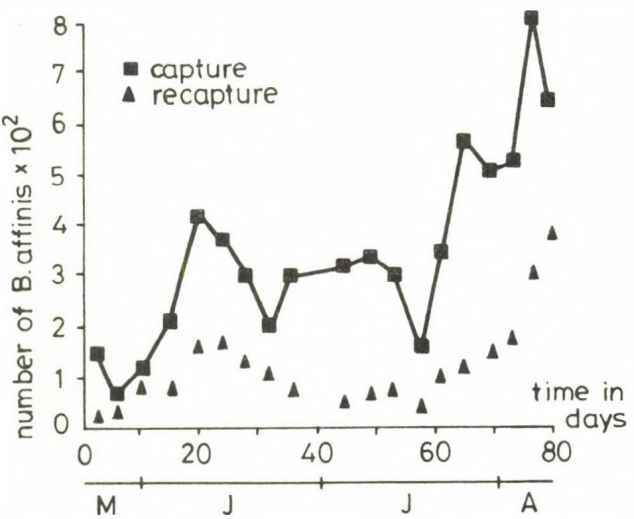

Fig. 3 B. affinis capture and recapture data at the time of *L. pratensis – L. sylvestris* flowering at St. Suzanne.

on July 5 in *L. sylvestris*. In both cases, the percentages of recapture are higher in *L. pratensis* than in *L. sylvestris* (Fig. 4).

Although the displacements were limited in space, some individuals were found far away from the plot of origin. For the whole period of study on *L. pratensis*, the maximum distance covered by an adult displacement during the 3/4 days interval was 80 m (June 15 from plot 6 to plot 2). It was 280 m (July 30 from plot 1 to plot 15) on *L. sylvestris*.

CONCLUSION

When they visit the flowers of *L. pratensis* or *L. sylvestris*, the adults of *B. affinis* can have an impact on pollination at three different levels: stigmatic receptivity, transfer of autopollen from the bottom of the keel to the stigma and transport of allopollen (Bashar et al., 1987). Because they spend the night in the flowers, it was suggested that the bruchid adults could live in a small territory and carry pollen to the flowers in the immediate vicinity of the inflorescence used as a dormitory. We now can state that although the displacements are reduced, the adults are capable

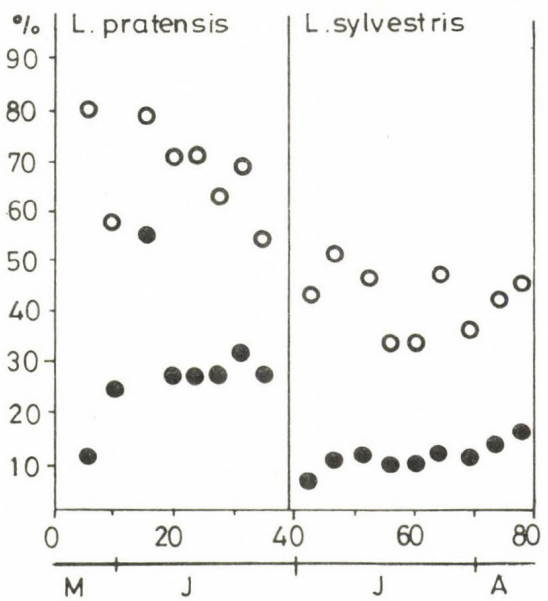

Fig. 4 Number of adults recaptured in the same plot compared to the total number of adults marked and released (solid circles) and to the total number of recaptured adults (empty circles).

of visiting the whole *Lathyrus* population and, eventually, other populations of the area.

The present study showed that the rate of recapture in the same plot was rather high. The adults of *B. affinis* usually stayed several days in the plot where they had been caught. This behaviour must be in relation to the abundance of flowers as we have seen a correlation between inflorescence density and the number of adults. But this moving behaviour is different in *L. pratensis* and in *L. sylvestris*. In the first case *B. affinis* visits the flowers only for pollen feeding. In the second case, pods are also visited for egg-laying. This observation shows that the behaviour of *B. affinis* adults changed after passing from the *L. pratensis* population to *L. sylvestris*. Two hypotheses can be proposed: 1) When the females are sexually mature, their foraging behaviour is different: they are still exclusively attracted to flowers but spend less time on them, passing from one inflorescence to the other more rapidly. Pods are found at random. 2) They are attracted to both flowers and pods. The search for pods of a certain quality, leads to longer displacement and a higher rate of dispersion on the *Lathyrus* population.

REFERENCES

Bashar, M. A., G. Fabres, and V. Labeyrie (1985): Stimulation of ovogenesis by flowers of *Lathyrus sylvestris* L. and *Lathyrus latifolius* L. in *Bruchus affinis* Frölich (Col. Bruchidae). In: A. K. Kaul and D. Combes (eds), *Lathyrus* and Lathyrism. Third World Medical Research Foundation, New York. 202-212.

Bashar, M. A., G. Fabres, M. Hossaert, M. Valero, and V. Labeyrie (1987): *Bruchus affinis* and the flowers of *Lathyrus latifolius*: an example of the complexity of relations between plants and phytophagous insects. In: V. Labeyrie, G. Fabres and D. Lachaise (eds), *Insects-Plants*. Dr. W. Junk Publ., Dordecht. 189-194.

Korab, I. I. (1927): The pea weevil problem. *Trud. Bulotzer. Selektz Sta. 11*, 81-126.

Southgate, B. J. (1979): Biology of Bruchidae. *Ann. Rev. Entomol. 24*, 449-473.

Wakeland, C. (1934): Flight studies of *Bruchus pisorum* L. *J. Econ. Ent. 27*, 534-542.

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RELATIONSHIPS BETWEEN *BRUCHUS RUFIMANUS* BOH. (COLEOPTERA: BRUCHIDAE) AND THE PHENOLOGY OF ITS HOST PLANT *VICIA FABA* (L.). THEIR IMPORTANCE IN THE SPATIAL DISTRIBUTION OF THE INSECTS

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ABSTRACT

Bruchus rufimanus (Coleoptera: Bruchidae) is a monovoltine specialist insect which develops in the green pods of *Vicia faba* (Leguminosae). Adults in reproductive diapause leave the hibernation sites when the host-plant begins to flower. Adults were sampled in the field by examining the nightly resting sites: in the flowers and young upper leaves. Displacement behaviour was studied by tracing individual adults during the day.

Displacement in the field is a spatio-temporal process linked to the heterogeneity of flowering, in terms of function of host plant density. The displacements of *B. rufimanus* are reduced in high flower density areas, since insects consume large quantities of *V. faba* pollen. When the number of flowering plants decreases, adults apparently seek areas where flowers are still available. At the end of the flowering period, displacements are still high and adults are observed on flowering weeds in and around the field.

The temporal structure of adult distribution influences the distribution pattern of eggs: they are laid primarily in areas where flowers are available. The importance of these behaviour patterns in terms of population dynamics is discussed.

Key words: Bruchidae, plant phenology, Leguminosae, insect displacements, insect feeding, egg-laying, egg distribution.

INTRODUCTION

Bruchus rufimanus (Boh) (Coleoptera: Bruchidae) is a phytophagous insect developing in Europe and in the Mediterranean countries on wild and cultivated Leguminosae of the genus *Vicia*. Females lay eggs on green pods and larvae develop inside the ripening pods. As most European species, *B. rufimanus* is a monovoltine insect which is in a reproductive diapause from the autumn to the onset of spring (Hoffmann et al., 1962). Adults hibernate in lichens or under the bark of trees, as observed in various monovoltine species of Bruchidae (Larson and Hinman, 1931; Wakeland, 1934; Genduso, 1978), and search for their host plants in the spring. They become sexually active at that time and reproduce on the green pods. In *B. pisorum*, the consumption of pollen of peas (Pajni and Sood, 1975) or other plant species (Larson et al., 1935; Pesho and Van Houten, 1982) is the main factor of

diapause termination. The new generation of adults emerges from the seeds during the summer and then reaches hibernation sites.

The aim of the present field work was to investigate the colonization conditions of a culture of broad beans (*Vicia faba* L.) by *B. rufimanus* adults and to analyze insect reproduction in the field.

MATERIAL AND METHODS

This research was carried out for three consecutive years (1986 through 1988) in the region of Vierzon (Center of France) in *V. faba* cultures. Fields between one and three hectares (2.5 to 7.5 acres) were divided into blocks and a given number of plants within each block was sampled. From May to July, they were observed every two or four days, depending on the year. The following parameters were thus studied:

1. *Changes in the plant.* The number of flowers and pods on the broad bean plants sampled and their position on the stems were noted at each observation.

2. *The number of adults in the plot.* Behavioural observations showed that during the scotophase the adults chose the young upper leaves and the flowers of broad beans as resting sites. They leave these sites in the morning when the temperature rises. Adults in the leaves and flowers on the sample plants were counted between 6 and 8 A.M. Adults captured were brought to the laboratory and they were :

- either released after sex determination,
- or dissected to determine the state of development of their reproductive organs and the contents of their digestive tract.

3. *Eggs laid on the pods.* All the pods on a sample plant were observed and the number of *B. rufimanus* eggs laid between two observations was noted. The eggs deposited on the pods were easily observed. They are glued to the pericarp by secretions, probably produced by the cells of the lateral oviducts, as in other Bruchidae species (Biémont et al., 1982).

RESULTS

Variations of B. rufimanus *adult number in a field of broad beans.* Adults were counted every two days in a field of broad beans (Soravi cultivar) divided into 72 blocks. During observations in each block, flowering was observed on one plant and adults were sought on five plants from may to July 1987.

Flowering begins in May and all the plants sampled had a mean of 20 to 30 flowers at the end of the month. Beginning in June, the mean number of flowers per plant and the percentage of plants sampled containing flowers decreases progressively. Flowering terminates at the beginning of July.

Table 1 Development of the adult reproductive organs captured in the *Vicia faba* culture during the four phases of the study.
A: From 13th to 20th May; B: From 20th May to 12th June.

	Phase 1	Phase 2		Phase 3	Phase 4
		A	B		
No of males	15	17	24	17	12
Sexually active males	15	17	24	17	12
No of females	25	20	22	23	21
Diapausing females	23	0	2	0	0
Oocytes in vitellogenesis	2	20	13	0	0
Mature oocytes	0	0	7	23	21

During the study, 2902 adults were captured on the plants sampled (sex ratio 0.55) but their number (Fig. 1), the development of their reproductive organs (Table 1) and their food (Table 2) varied with time.

The following four phases of plant development were distinguished:

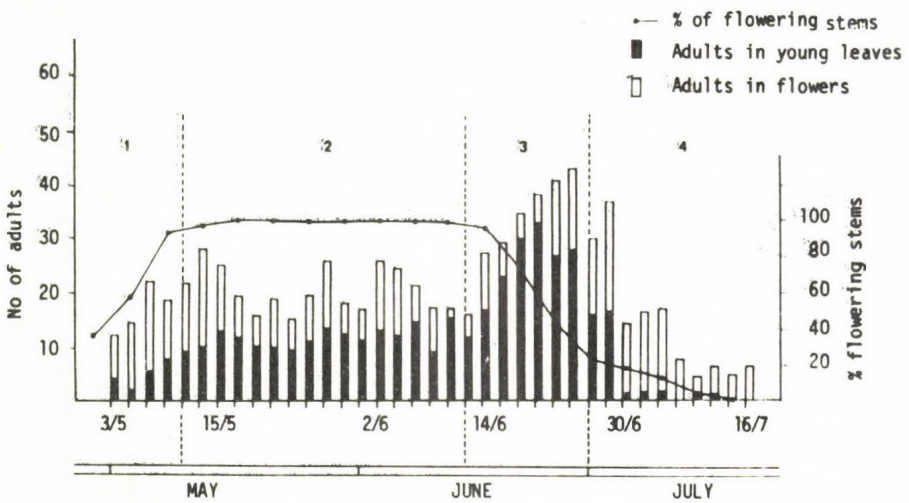

Fig. 1 Variations in the number of *B. rufimanus* adults captured in the *V. faba* culture from 29th April 1987 to 16th July 1987 and in the percentage of flowering stem.

Table 2 Study of the pollen observed in the *B. rufimanus* digestive tract of adults captured during the four phases of the study inside the *Vicia faba* culture.
nd: species not determined

Phases	1	2	3	4
Pollens observed in the digestive tract	*Vicia faba* *Salix alba* Cruciferae (nd)	*Vicia faba*	*Vicia faba* *Papaver rhoeas* *Sisymbrium irio* *Heracleum sphondylium* *Tilia* sp. Compositae	*Vicia faba* *Lathyrus tuberosus* *Sisymbrium irio* *Heracleum sphondylium* Compositae (nd) *Lotus corniculatus* *Galium cruciata* *Convolvulus sepium*

1. *At the beginning of flowering* (from April 29 to May 11) 462 adults were captured, of which 67% were on young leaves. All the males dissected during this period had functioning reproductive organs, whereas the females were still in reproductive diapause. Oocytes in previtellogenesis were observed at the base of their ovarioles, but no phases of vitellogenesis was observed. No female was inseminated. The digestive tract of captured adults contained the pollen of broad beans and of other flowering plant species.

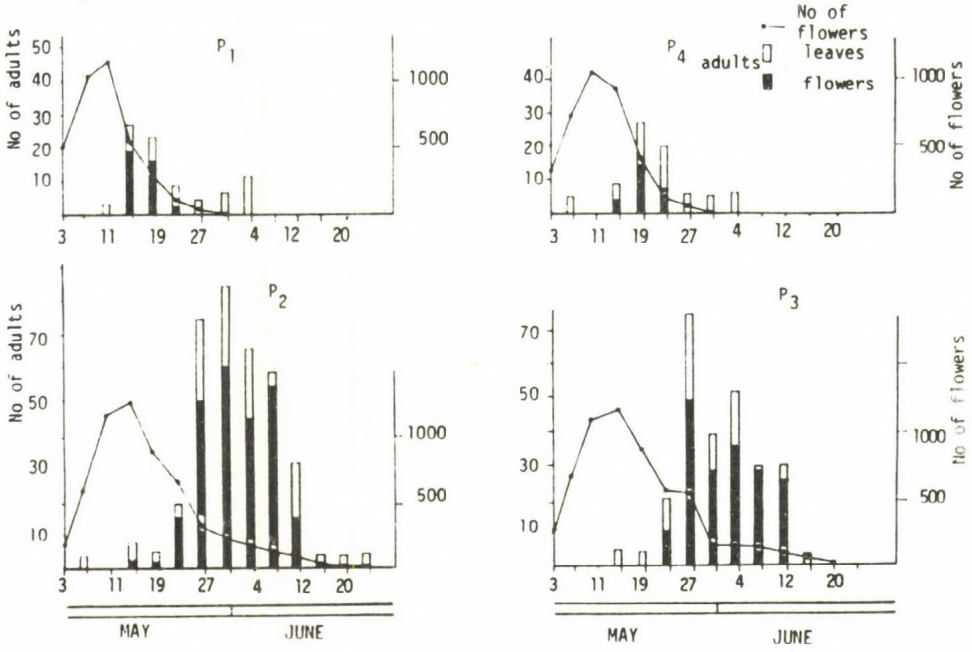

Fig. 2 Variations in the number of *B. rufimanus* adults captured in the four plots from May to June 1988 and in the number of flowers.
P_1 (18 plants/m^2) and P_4 (27 plants/m^2) = densely planted plot
P_2 (8 plants/m^2) and P_3 (9 plants/m^2) = sparsely planted plot

Table 3 Estimation of *B. rufimanus* egg-layings on the sampled pods inside the four plots in 1988

Plots	Pods without seeds			Pods with seeds		
	No	%of pods with eggs	Mean number of eggs/pods	No	%of pods with eggs	Mean number of eggs/pods
P1	512	3.3	1.3	490	53.7	2.3
P2	731	10.1	1.7	1016	57.3	2.9
P3	724	6.6	0.9	907	38.8	2.4
P4	405	3.7	0.7	535	39.1	1.8

2. *At the height of flowering* (from May 13 to June 12), 295 adults were found in the flowers or the young leaves, but the number of insects captured and the predominant sites of capture varied with the date. Captured males were sexually active. In females, vitellogenesis began slowly and mature oocytes were observed in the lateral oviducts only after May 20. Adults consumed only broad bean pollen during this period.

3. *During the decline of flowering* (from June 14 to 26), the number of insects captured in the flowers increased and reached a maximal value on June 20. During this period five or six adults could be found in the same flower in the morning. The increase in captures inside the flowers is probably due to a gathering of adults in the last available resting and feeding sites. The reduction of the number of flowers results in a diversity of food. The insects consume the pollen of other flowers available in the agrosystem.

4. *At the end of flowering* (from June 28 to July 16) when a limited number of flowers were present in the field, the number of insects captured (only on young leaves) decreased. During this phase, the bruchids consumed the pollen of a large number of plants flowering at that time. *B. rufimanus* may even seek nocturnal resting sites in the flowers of another Leguminosae, *Lathyrus tuberosus*.

The monitoring of adults thus shows the importance of the availability of food resources on the behaviour of the insects, and probably on their spatial distribution within the plot as well. From May to June 1988, adults were followed in four small plots inside the same field, differing by plant density. Increasing plant density resulted in a decreased number of flowers (Fig. 2), as previously observed by Hodgson and Blackman (1955) and in a reduced period of flowering. Beginning at the end of May, the broad bean in the dense plots (P1, P4), no longer produced flowers. In sparsely planted plots (P2, P3), however, flowering continued until the end of June. The monitoring of adults (Fig. 2) showed that the colonization of the field began in the dense plots: 68% of the captures between May 15 and 23 were at this level. The regression in the number of flowers formed in P1 and P4 was accompanied by a modification of the spatial distribution of adults. At this time, there was an increase in the number of captures on plants in the low density plots P2, P3 : 94% of the captures between May 27 and June 12 were at this level.

As before, the decreased number of flowers available at the end of June in plots P2 and P3 caused a gathering of adults on the last plants still flowering, followed by a regression in the plot, since the insects sought other Leguminosae still flowering in the agrosystem.

Egg-layings on the V. faba *pods.* The eggs laid by *B. rufimanus* were followed in the four plots (P1, P2, P3, P4) inside the field from May to July 1988. The consequences of variations in adult density at the resting site were examined on the egg-distribution on the pods produced by the stems.

At harvest (Table 3) the sampled stems in the densely planted plots matured fewer pods than those in the sparsely planted plots, but in all cases 50% of them produced no seeds. These sterile pods received 3 to 10% of the eggs laid in each plot. The females preferentially laid eggs on pods with seeds but the percentages of pods with eggs were highly variable depending on the plot.

The analysis of eggs laid on pods with seeds in P1 and P2 plots (Fig. 3) show that eggs were laid in each plot from May to June even when the density of adults in the resting sites was low.

Egg-laying began on pods in the dense P1 plot containing the most precocious broad beans, reaching a maximal value on the 23th May, then decreasing. The appearance of eggs on the pods in the sparsely planted plot (P2) occurred later: the percentages of pods with eggs reached maximal values at the end of May. The high number of eggs laid during this period could be the result of changes in the spatial distribution of adults. At this time, flowering in P1 was terminated and adults sought flowers still available in the field (as in P2 plot). They probably laid most of their eggs on pods near the last flowering stems where they found feeding and resting sites.

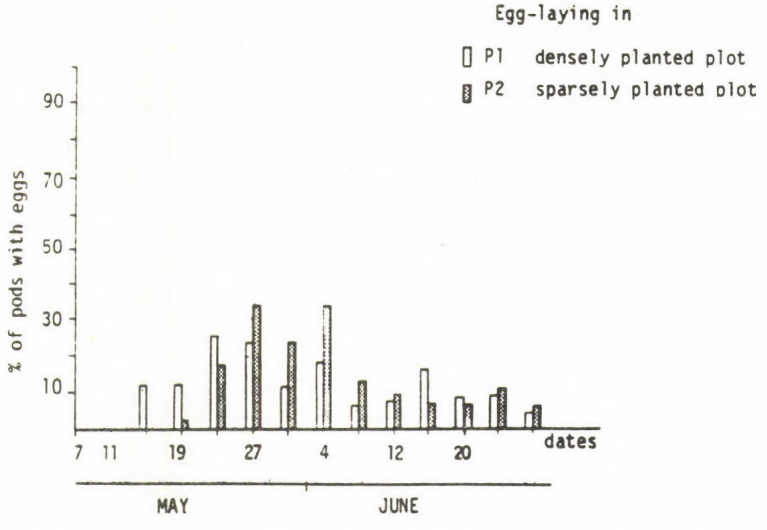

Fig. 3 Percentages of pods receiving eggs at each observation during May and June

After the end of the flowering period, in June, 5 to 10% of the pods still received eggs at each observation in both plots.

DISCUSSION

Observations have shown that the colonization of the field of broad beans by *B. rufimanus* adults begins during the phase of plant flowering. The conditions of migrations from the hibernation site to the fields are unknown, but it is probable that climatic factors play an important role, as observed in Coccinellidae (Hagen, 1962; Hodek, 1973) or with *B. pisorum* (Pesho and Van Houten, 1982). The reproductive organs of males are developed at the beginning of the colonization phase, when the females are still in reproductive diapause. Observations (Tran and Huignard, unpublished) have shown that the consumption of pollen of various flowering plant species at the beginning of spring is sufficient to induce the sexual maturation of the males. The presence of flowers of the genus *Vicia* (and probably the consumption of the pollen of this plant) is necessary for the induction of vitellogenesis.

Behavioural observations (Dupont, 1989) have shown that adults in the field devote a large part of their time to visiting flowers and to consume pollen. According to the "resource concentration" hypothesis of Root (1973), when the quantity of available food is large, this would maintain the adults in the field and they would consume only the pollen of their host plant. The decreased number of flowers would lead to a reduced quantity of available food and also reduce the number of potential nocturnal resting sites. This decrease would thus have important effects on *B. rufimanus* adults. These would be displacements within the field, since insects would seek out plants still bearing flowers. This phenomenon would explain the gathering in the last available flowers and the changes of the feeding behaviour, the insects becoming more generalists. As in other phytophagous insects studied by Bach (1980) and Kareiva (1983), the decrease of the quantity of available food led to an increased mobility of adults.

B. rufimanus egg-layings are observed on pods from May to July but the percentages of pods receiving eggs were variable and depending on the plots in the culture and on the time. Displacements of adults inside the plots, especially during the search for resting sites, may influence egg distribution. Thus, at the end of May, when the adults tend to use the flowers formed in the sparsely planted plots as resting sites, there is an increased frequency of egg laying at this level. It is nevertheless not possible to establish a precise relationships between these two phenomena, since the behaviour of adults during the day inside the field is poorly known.

The spatial distribution of eggs, in fact, depends on the interaction of factors where the structure of the pods (absence or presence of seeds), the date of pod appearance on the stem and the number of precocious pods available on the stems also influence the female's oviposition behaviour (Dupont, 1989). The decrease of the number of eggs laid on the pods at the end of June could be due to a reduction of the adult numbers or to a limitation of their reproductive activity (because of

reduced food supply). The high number of pods available in the field during this month could also influence the egg distribution as observed in other insects (Singer, 1982; Rauscher, 1983; Bach, 1984).

REFERENCES

Bach, C. E. (1980): Effects of plant density and diversity on the population dynamics of a specialist herbivore, the stripped cucumber beetle *Acalymma vittata*. *Ecology 61*, 1515-1530.

Bach, C. E. (1984): Plant spatial pattern and herbivore population dynamics: plant factors affecting the movement pattern of a tropical cucurbit specialist *Acalymma vittata*. *Ecology 65*, 175-190.

Biemont, J. C., G. Chauvin and J. F. Germain (1982): L'oeuf de *Bruchidius atrolinatus* et son système de fixation. *Can. J. Zool. 60*, 2610-2615.

Dupont, P. (1989): Contribution à l'étude des populations de *Bruchus rufimanus* (Boh.). Influence de la phénologie de la plante hôte *Vicia faba* sur la répartition spatiotemporelle des adultes. *Thèse Doctorat Université de Tours.*

Genduso, P. (1978): Insectes nuisibles aux Légumineuses en Sicile et observations sur l'hivernage des bruches monovoltines. *Boll. Int. Entomol. Agr. Oss Fitopat. Palermo, 10*, 169-175.

Hagen, K. S. (1962): Biology and ecology of the predaceous Coccinellidae. *Ann. Rev. Entomol. 7*, 289-326.

Hodek, I. (1973): *Biology of Coccinellidae*. Academia Czechoslovakia Publ.

Hodgson, G. L. and G. E. Blackman (1955): An analysis of the influence of plant density on the growth of *Vicia faba*. I. The influence of the density on pattern of development. *J. Exp. Bot. 7-20*, 147-165.

Hoffmann A. and V. Labeyrie, A. S. Balachowsky (1962): Famille des Bruchidae. In: A. S. Balachowsky (ed), *Entomologie Appliquée à l'Agriculture*. Masson Publ, Paris. 434-494.

Kareiva, P. (1983): Influence of the vegetation texture on herbivore populations in resource concentration and herbivore movements In: R. F. Denno and M. S. McLure (eds), *Variable plants and herbivore in natural and managed systems.* Academic Press, New York. 259-289.

Larson, A. O. and F. G. Hinman (1931): Some hibernation habits on the pea weevil in relation to its control. *J. econ. Entomol. 24*, 965-968.

Larson, A. O., T. A. Brinley, and F. G. Hinman (1935): Some recent additions to our knowledge of the biology of the pea weevil. *J. econ. Entomol. 28*, 668-670.

Pajni, H. R. and S. Sood (1975): Effect of pea pollen feeding in maturation and copulation in the beetle *Bruchus pisorum*. *Ind. J. Exp. Biol. 13*, 202-203.

Pesho, G. R. and R. J. van Houten. (1982): Pollen and sexual maturation of the pea weevil. *Ann. Entomol. Soc. America 75-4*, 439-443.

Rauscher, R. B. (1983): Alteration of oviposition behaviour by *Battus philenor* butterflies in response to variation in host plant density. *Ecology 64-3*, 1028-1034.

Root, R. B. (1973): Organization of a plant arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecol. Monogr. 43*, 95-124.

Singer, M. C. (1982): Quantification of host preference by oviposition behaviour in the butterfly *Euphydryas editha*. *Oecologia 52*, 224-229.

Wakeland, C. (1934): Flight studies of *Bruchus pisorum* L. (Coleoptera, Bruchidae). *J. econ. Entomol. 27*, 534-542.

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WHAT ARE GALLS FOR? TESTS OF THE NUTRITION HYPOTHESIS

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ABSTRACT

Galls are remarkably close associations between arthropods and plants, in which the presence of an insect or mite causes already differentiated plant tissue to be changed radically. The insect becomes the new organizer of plant development, and modifies the plant's growth and metabolism to produce often quite bizarre structures.

A question which has long puzzled biologists is: what are galls for? What is the adaptive significance of this enclosed and immobile lifestyle? One possibility is the Nutrition Hypothesis which suggests that the concentration of nutritive compounds (such as proteins and sugars) are higher in galls than in host plant tissue, whilst the levels of defensive compounds (e.g. phenolics) are lower. I have examined this hypothesis in two experiments.

In the first experiment many different gall species, from a range of different plants, were collected. The galls, adjacent plant tissue, and appropriate undamaged tissue were analysed for nitrogen, sugars and phenolics. The results were rarely as predicted by the theory, but allowed some insights into the nature of the insect-plant interaction.

The second experiment was a more direct test of the hypothesis, and asked: can the gall-insect manipulate host-plant nutrients, as predicted by the theory, and if so does it benefit as a result? The experiment monitored the effect of fertilizing oak trees on the relative nitrogen content of a common oak gall, *Neuroterus quercus-baccarum* (Cynipidae), and on the oviposition rate, growth rate and overwinter survivorship of the insects. Results so far are equivocal, but this sort of experiment may be the most direct way to test the Nutrition Hypothesis.

Key words: Plant galls, cynipids, *Neuroterus quercus-baccarum*, *Quercus robur*, nitrogen, phenolics, insect nutrition, host-plant manipulation, adaptive behaviour.

INTRODUCTION

Galls are remarkably close associations between arthropods (usually insects) and plants. There are at least 15,000 spp. of gall-inducing insects, in only a few insect orders. Generally they are host-specific (Rofritsh and Shorthouse, 1982); each gall-former inducing galls with distinct characteristics (Felt, 1965; Meyer, 1987), in which already differentiated plant tissue is changed markedly by an insect-derived stimulus (Shorthouse and Lalonde, 1988). The major hypotheses put forward to explain the adaptive nature of galls have been recently reviewed by Price et al. (1986, 1987). They are:

Table 1 The gall and plant species collected for analysis in Expt 1.

Spp. number	Gall species	Order	Plant species
1.	*Pontania viminilis*	Hymenoptera	*Salix caprea*
2.	*Pontania proxima*	Hymenoptera	*Salix alba*
3.	*Iteomyia capreae*	Diptera	Salix caprea
4.	*Pemphigus spirothecae*	Homoptera	*Populus nigra*
5.	*Eriophyes macrorhynchus*	Acarina	Acer pseudoplatanus
6.	*Eriophyes laevis inangulis*	Acarina	*Alnus glutinosa*
7.	*Eriophyes gonithoraxtypicus*	Acarina	*Crataegus monogyna*
8.	*Eriophyes similis*	Acarina	Prunus spinosa
9.	*Diplolepis rosae*	Hymenoptera	Rosa canina
10.	*Diplolepis eglanteriae*	Hymenoptera	*Rosa canina*
11.	*Cynips divisa*	Hymenoptera	Quercus robur
12.	*Neuroterus quercus-baccarum a) asexual generation b) sexual generation*	Hymenoptera	Quercus robur
13.	*Biorhiza pallida*	Hymenoptera	*Quercus robur*
14.	*Andricus lignicola*	Hymenoptera	*Quercus robur*
15.	*Andricus fecundator*	Hymenoptera	*Quercus robur*
16.	*Andricus quercuscalicis*	Hymenoptera	*Quercus robur*

i) Microenvironment Hypothesis (Fernandes and Price, 1988). Galls provide the insect with shelter from hygrothermal stress.

ii) The Enemy Hypothesis (Price and Pschorn-Walscher, 1988; but see Hawkins, 1988). Galls provide protection from parasitoids.

iii) The Nutrition Hypothesis (e.g. Bronner, 1977). Galls provide a better nutrient source than other plant tissue. This third hypothesis is the subject of the present paper.

There is some evidence that galls contain higher nutrients e.g. sugars, lipids, proteins and amino-acids and lower plant secondary compounds than ungalled tissue (Paclt and Hassler, 1967; Shannon and Brewer, 1980; Cornell, 1983). But not all studies have found this (Brewer et al., 1987; Hawkins and Unruh, 1988). There is similar confusion over whether galls are active nutrient sinks, i.e. attract more nutrients than an equivalent weight of ungalled plant tissue (Jankjewicz et al., 1970). Furthermore, most of this evidence is weak since it is correlational; the problem is that the nutritional requirements of gall-formers are largely unknown. The question which needs to be addressed is "does alteration of nutrient levels in the gall really improve nutritional quality for gallers?". So far there have been few experiments designed to test such ideas.

The present study examines whether the prediction of higher nutrients and lower defensive compounds in galls compared to other plant tissue is widely applicable, by analysing a wide range of plant and gall species. Then I examine whether increased nutrients do benefit gallformers by measuring the performance of two species on trees with elevated nutrient levels.

Table 2 The levels of nitrogen, phenolics and carbohydrate (% dry weight) in galled (G) and equivalent ungalled (U) tissue from 16 species of galls (see Table 1).

Species		Nitrogen			Phenolics			Carbohydrate		
		G	U	p	G	U	p	G	U	p
1	Y	2.16	1.81	ns	14.3	10.7	ns			
	M	0.82	1.56	*	10.1	12.7	*	23.1	10.8	**
2	Y	2.71	2.63	ns	16.0	8.1	**			
	M	0.77	1.56	ns	15.8	10.2	**	36.8	13.5	***
3	Y	1.04	1.70	**	13.9	12.0	ns			
	M	0.74	2.14	**	8.8	13.9	*	34.9	13.3	**
4	M	1.86	0.89	NS	13.1	3.4	***			
5	M	0.36	1.07	**	18.6	15.9	ns	7.7	4.8	ns
6	M	0.54	1.91	**	22.7	8.1	***	11.2	8.8	ns
7	Y	1.94	2.26	ns	17.3	11.7	**			
	M	0.48	0.71	ns	13.2	9.8	*	12.2	8.4	ns
8	Y	2.60	2.92	ns	9.7	8.5	**			
	M	0.39	1.36	ns	18.8	9.5	***	17.9	8.7	***
9	Y	0.82	1.36	*	51.0	16.4	***	4.8	10.1	ns
10	M	1.01	2.40	*	10.9	20.0	***			
11	Y	0.59	1.72	*	7.6	10.9	ns			
	M	0.90	1.94	ns	9.6	16.1	**			
12	Y	1.79	2.02	ns	18.1	11.9	*	8.9	8.1	ns
a)	M	0.81	2.18	***	21.3	16.8	*			
b)	Y	1.05	2.15	*	4.0	10.0	**			
13	Y	2.84	2.32	ns	20.2	14.3	**			
	M	2.43	2.12	ns	10.8	14.0	ns			
14	M	1.45	2.37	**	33.3	12.3	***			
15	M	1.82	3.04	***	25.9	19.9	**			
16	M	1.32	1.37	ns	18.1	10.9	***			

Y=young and M=mature galls. * indicates significance levels (p) in oneway ANOVA of galled vs. ungalled tissue (*=p;0.05; **=p<0.01; ***=p<0.001; ns=non-significant). The carbohydrate analysis is incomplete.

EXPERIMENT 1

Methods. In 1988 16 species of galls growing on 9 species of plants (Table 1) were collected; where possible both young and mature galls were sampled. Galled and equivalent ungalled tissue was analysed for total nitrogen (Hinds and Lowe, 1980) total phenolics (Hartley and Firn, 1989) and total carbohydrate (Jermyn, 1975). Gall insects were removed before analysis and if the galls were large enough only the inner layer of tissue was analysed. There were four replicates per sample.

Results. The results of the nitrogen, sugar and phenolic analysis are shown in Table 2. These were initially analysed by a two-way ANOVA (galling gall species). Nitrogen, sugar and phenolics were analysed separately, as were young and mature galls. For

Table 3 The number of cases which obey simple predictions of the nutrient hypothesis for the relative levels of chemicals in galled (G) and ungalled (U) tissue.

Chemical	Prediction	As predicted	Not as predicted	
Nitrogen	G>U	0	25	(12 G=U; 13 G<U)
Phenolics	G<U	5	20	(5 G=U; 15 G>U)
Carbohydrate	G>U	4	5	(5 G=U; 0 G<U)

all three chemical characteristics, both species and galling had a major effect ($p<0.001$ in all tests), but in each case the interaction between galling and species was also highly significant ($p<0.01$ in all cases). A significant interaction term suggests, for example, that gall nitrogen can be greater or less than the level in ungalled tissue depending on which gall-forming species is being considered. Thus it was decided to test for an effect of galling on nutrient levels for each species separately, using one-way ANOVA's (Table 2).

Discussion. Table 2 shows clear differences between galled and ungalled tissue in terms of all three chemicals. Furthermore, arthropod species behave differently from each other, even when they gall the same plant species, as do young and mature galls. Taken at their face value, these data do not fit well with the prediction of the nutrient theory. For example, if nitrogen is a limiting nutrient for most phytophages (Mattson, 1980), the hypothesis predicts that nitrogen content should be elevated in galls, but this was not found in any of 25 cases. Similarly phenolic content (usually assumed to be detrimental to phytophages) was lower in galls in only 5 cases. Only carbohydrate content was generally higher in galled tissue (Table 3).

However, we need to interpret these data carefully. For example, key nutrient could be restricted to a very narrow layer of tissue, which the techniques used here may not measure. Similarly, very specific nutrients may be elevated (e.g. particular amino acids), which again crude methods like "total nitrogen" might not detect. The most serious problem however, is that the experiment is an indirect test of the nutrient theory because the data are correlational. The nutrient theory requires not just raised concentrations of beneficial compounds and reduced levels of harmful ones in the gall, but more specifically that the insects do better than they would if they fed on tissues more similar in composition to the rest of the plant. More powerful tests of the theory can be made available to the plant and insect performance monitored directly. The crucial questions are:

1. Does increasing host nitrogen lead to increased nitrogen content in the gall, in a way that suggest the gall is an active sink, i.e. gains a greater proportion of plant nutrients?

2. Does the gall insect benefit from such manipulation, e.g. in terms of faster growth rate or better survivorship?

These predictions were tested in the second experiment.

EXPERIMENT 2

Methods. The system chosen was oak (*Quercus robur*); 16 trees (2-5 m tall) were selected, and fertilizer was applied monthly (starting on 11/3/88) to 8 of the trees, at a cumulative annual dose of 10 g/m. Two gall species were sufficiently abundant for a comparison of nitrogen content in galled and ungalled tissue: *Andricus lignicola* and *Neuroterus quercus-baccarum* (asexual generation). At the start of the experimental period (26/7/88) and later in the season (16/9/88) 40 *Neuroterus* galled leaves, and 20 ungalled leaves were collected from each tree, whilst on 28/9/88 *A. lignicola* galls and ungalled tissue were collected (4 replicates only). The samples were analysed for nitrogen as in Expt 1. Only *Neuroterus* was abundant enough for further measurements; it's performance on fertilized and unfertilized trees was measured in 4 ways:

1. The number of galls on 50 leaves was counted; the mean density of galls/leaf at the beginning of the growing season provided a measure of oviposition preference by adults of the parent generation.

2. The growth rate of galls on each tree was measured by collecting 5 leaves at weekly intervals (from 26/7/88) and weighing 4 insects/leaf to produce a mean dry weight for each date.

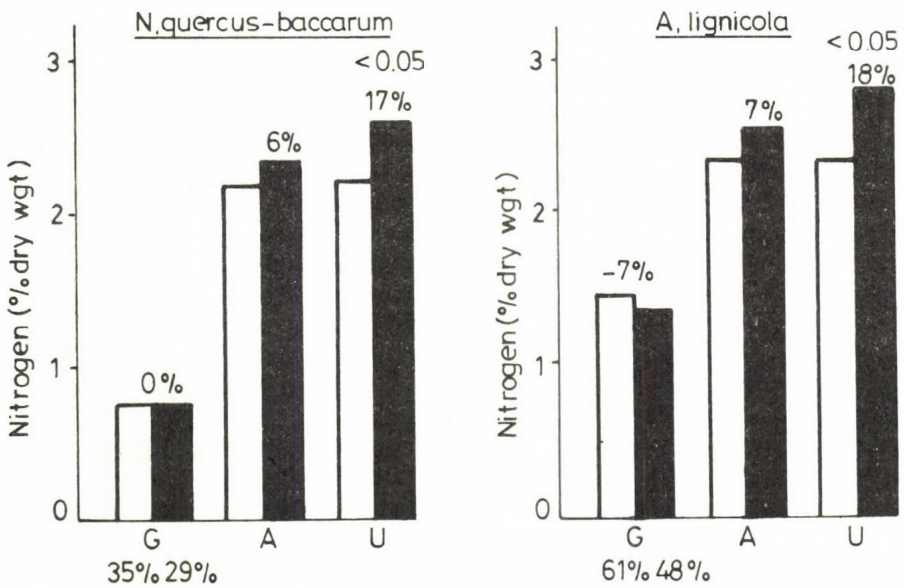

Fig. 1 The nitrogen content (% dry weight) of galls (G), the ungalled part of galled leaves (A) and ungalled leaves (U) for two species of gall-former. Shaded columns represent samples from fertilized trees, unshaded from unfertilized ones; the percentages above the columns indicate the difference between fertilized and unfertilized trees, and < 0.05 means the difference is significant. The gall nitrogen level as a percentage of host plant nitrogen is also shown.

3. Survivorship was measured as the proportion of gall insects successfully emerging in spring 1989. At the end of the season (5/10/88) 50 galled leaves were collected from each tree, the galls removed (up to a maximum of 500) and placed on damp sand in flasks in an outdoor insectary.

4. The weight of emerging adults (all female) was measured after they were all air-dried; 30 randomly selected individuals were weighed.

Results. Does increasing plant nitrogen lead to a greater proportion of nitrogen in the galls? The results of the nitrogen analysis suggest the opposite (Fig. 1). Fertilising the trees does increase nitrogen levels in the ungalled leaves (*Neuroterus* trees: $t = 2.2$, $df = 14$, $p = 0.045$; *A. lignicola* trees: $t = 2.5$, $df = 6$, $p = 0.047$). But in ungalled tissue from galled leaves the increase was much smaller, and in the galls themselves the nitrogen levels were identical on fertilized and unfertilized trees. In fact both species of gall are attracting less nutrients on the high nitrogen trees, and the insect is receiving a *smaller* proportion of the host-plant's nitrogen as the plant's nitrogen level rises.

Do the gall insects do better on high nitrogen trees? Fertilizing the host plant does not seem to bring any benefit to the gall insects. Mean gall densities were no

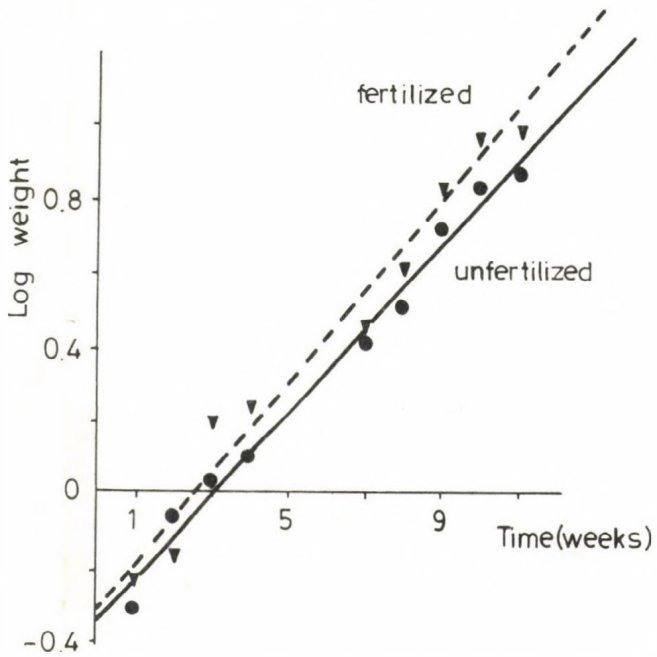

Fig. 2 The growth rates (log mean dry weight in ug vs time in weeks) of *Neuroterus quercus-baccarum* growing on fertilized (▼) and unfertilized (●) trees. On fertilized trees the regression equation is: log weight = -0.305 + 0.121 time; $r^2 = 96.8\%$; on control trees log weight = -0.337 + 0.112 time; $r^2 = 98.5\%$. The slopes of the regression lines do not differ significantly ($t = 0.91$, $df = 14$, $p = 0.1$).

Table 4 The proportion of adults emerging on each tree and the mean dry weight of 30 individuals.

Tree	% emerging	mean dry weight (mg)
U1	10	0.1439
U2	13	0.1574
U3	22	0.1466
U4	6	—
U5	28	0.1263
U6	4	—
U7	87	0.1902
U8	2	—
F1	67	0.1584
F2	40	0.1985
F3	3	—
F4	13	0.1321
F5	6	—
F6	72	0.2162
F7	36	0.1642
F8	14	0.1318

U=unfertilized trees, F=fertilized trees

main effect	df	SS	MS	F	p
TREE	4	5.148	1.287	5.26	0.001
FERTILIZING	1	0.519	0.519	2.12	0.146
error	294	71.980	0.245		

A two-way ANOVA of log weight against effect of tree and effect of fertilizing was performed.

different on fertilized and unfertilized trees (13 and 14 galls/leaf respectively), and growth rates were no faster on fertilized trees (Fig. 2). Similarly, although survivorship varied geatly across the trees, the proportions successfully emerging did not differ between fertilized and unfertilized trees (Table 4; $t = 0.75$, $df = 14$, $p = 0.1$). The mean weight of the adults also varied with tree (Table 4) and a two-way ANOVA of log weight confirms the large variation between trees, but finds no effect of fertilizing.

Given this between-tree variation, some of the data were reanalysed by correlation, taking the trees as a whole. Oviposition preference and adult weight were both correlated with survivorship, and gall nitrogen content was *inversely* correlated with survivorship (Fig. 3).

Discussion. At first sight the data do not support the nutrient theory. A simple interpretation of theory predicts that galls should be active nutrient sinks and accumulate nitrogen preferentially. In fact, on fertilized trees the gall's relative nitrogen

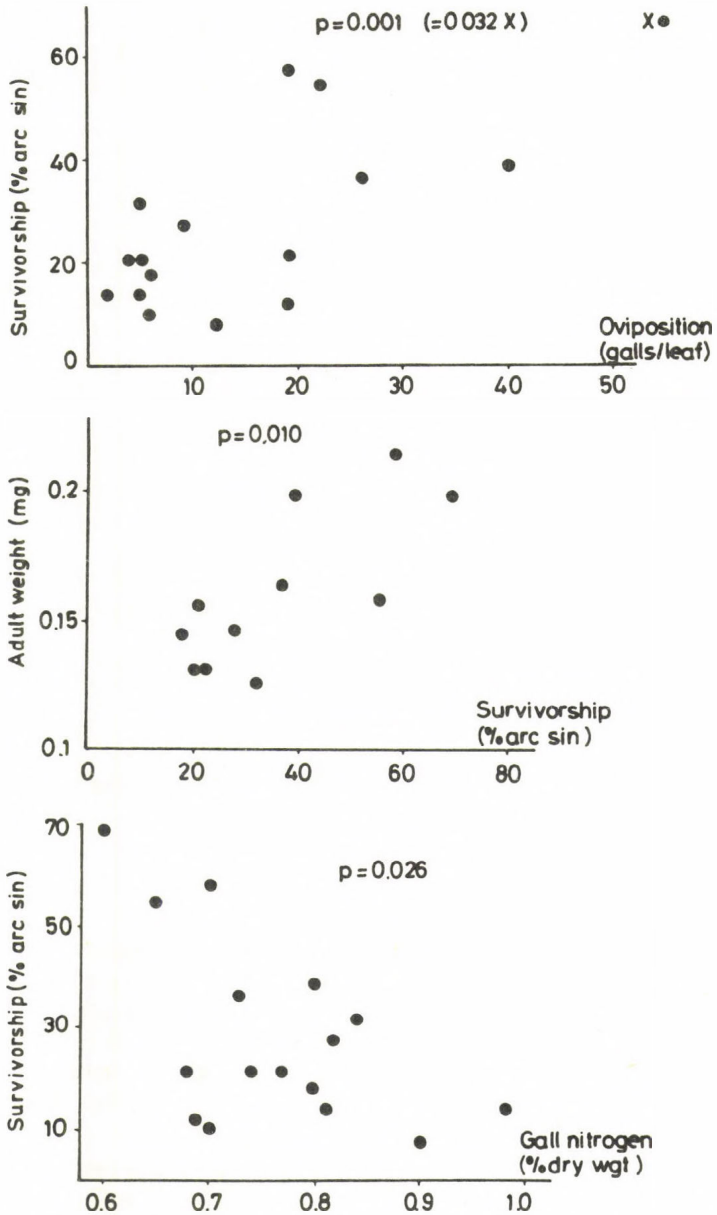

Fig. 3 Correlation analysis using both fertilized and unfertilized trees. The significance level of the correlation is shown.

a) Oviposition preference (galls/leaf) vs survivorship (% adults emerging, arc sin transformed). the significance level with the outlier (X) excluded is also shown.

b) Survivorship (% arc sin transformed) vs. adult weight (mg).

c) Gall nitrogen (% dry weight) vs. survivorship (% arc sin transformed).

content goes down; not surprisingly then, growing on high nitrogen hosts provides no benefit in terms of growth rate, survivorship or adult weight.

However, if the nutrient theory is restated more generally, in terms of adaptive behaviour i.e. "galls allow the insect to manipulate plant nutrient for their own benefit", then the theory is supported by this experiment, since this is precisely what *Neuroterus* appears to do. The females oviposit on the trees which give the best survivorship and the largest adults. Larvae in the galls then attract relatively less nitrogen compared to the host, but again this would appear to be adaptive behaviour since lower gall nitrogen content gives better survivorship! Thus the insects *do* appear to manipulate plant nutrients and apparently benefit as a result. The odd thing is that the manipulation is in the "wrong" direction! However, there are a small but growing number of examples where lower host-plant nitrogen produces better larval performances (e.g. Strong et al., 1984). Furthermore, the adaptive behaviour need not be the same for all species, e.g. Abrahamson and McCrea (1986) found that fertilizing galled plants did increase the nitrogen levels in the galls, but this produced no effect on gall weight and they concluded that this species was not nitrogen limited.

In conclusion, this experiment does support the nutrient theory, but it is not necessarily *more* nutrients that the gall-former requires; since nutritional requirements are likely to vary with different species of gall-formers, so should the predictions of the nutrient theory. The highly significant interaction terms found in Expt. 1 may therefore represent very precise manipulations of host-plant chemistry by each species of insect.

REFERENCES

Abrahamson, W. G. and K. D. McCrea (1986): Nutrient and biomass allocation in *Solidago altissima*: effects of two stem gallmakers, fertilization and ramet isolation. *Oecologia 68,* 174-180.

Brewer, J. W., J. N. Bishop and V. Skuhravy (1987): Levels of foliar chemicals in insect-induced galls. *J. Appl. Entomol. 104,* 504-510.

Bronner, R. (1977): Contribution à l'étude histochimique des tissu nourriciers des zoocécidies. *Marcellia 40,* 1-134.

Cornell, H. V. (1983): The secondary chemistry and complex morphology of galls formed by the Cynipidae (Hymenoptera): why and how? *Am. Midl. Nat. 110,* 225-234.

Felt, E. P. (1965): *Plant Galls and Gall Makers*. Hafner, New York.

Fernandes, G. W. and P. W. Price (1988): Biogeographical gradients in galling species richness. *Oecologia 76,* 161-167.

Hartley, S. E. and R. D. Firn (1989): Phenolic biosynthesis, leaf damage, and insect herbivory in birch (*Betula pendula*). *J. Chem. Ecol. 15,* 275-283.

Hawkins, B. A. (1988): Do galls protect endophytic herbivores from parasitoids? A comparison of galling and non-galling Diptera. *Southw. Nat. 13,* 473-474.

Hawkins, B. A. and T. R. Unruh (1988): Proteins, water and the distribution of *Asphondylia atriplicis* (Diptera: Cecidomyidae). *Southw. Nat. 33,* 114-117.

Hinds, A. A. and L. E. Lowe (1980): Application of the Berthelot reaction to the determination of ammonium nitrogen in soil extracts and soil digests. *Comm. Soil. Sci. Pl. Anal. 11,* 469-475.

Jankjewicz, L. S., H. Plich and R. Antoszewski (1970): Preliminary studies on the translocation of carbon-14 labelled assimilates and phosphate-32 towards the gall evoked by *Cynips quercus-foli* on oak leaves. *Marcellia 36,* 163-172.

Jermyn, M. A. (1975): Increasing the sensitivity of the anthrone method for carbohydrate. *Anal. Biochem. 68,* 332-335.

Mattson, W. J. (1980): Herbivory in relation to plant nitrogen content. *Ann. Rev. Ecol. Syst. 11,* 119-161.

Meyer, J. (1987): *Plant Galls and Gall-Inducers*. Borntrager, Berlin.

Paclt, J. and J. Hassler (1967): The concentration of nitrogen in plant galls. *Phyton. Aust. 12,* 173-176.

Price, P. W., G. L. Waring and G. W. Fernandes (1986): Hypotheses on the adaptive nature of plant galls. *Proc. Entomol. Soc. Wash. 88,* 361-363.

Price, P. W., G. W. Fernandes and G. L. Waring (1987): The adaptive nature of plant galls. *Environ. Entomol. 16,* 15-24.

Price, P. W. and H. Pschorn-Walcher (1988): Are galling insects better protected against parasitoids than exposed feeders? A test using tenthredinid sawflies. *Ecol. Entomol. 13,* 195-205.

Rofritsh, O. and J. D. Shorthouse (1982): Insect galls. In: G. Kahl and J. S. Schell (eds), *Molecular Biology of Plant Tumours*. Acad. Press, New York. 131-152.

Shannon, R. E. and J. W. Brewer (1980): Starch and sugar levels in three coniferous insect galls. *Z. angew. Entomol. 89,* 526-533.

Shorthouse, J. D. and R. G. Lalonde (1988): Role of *Urophora cardui* (Diptera: Tephritidae) in growth and development of its galls on stems of Canada thistle. *Can. Entomol. 120,* 639-646.

Strong, D. R., J. H. Lawton and R. Southwood (1984): *Insects on Plants: Community Patterns and Mechanisms*. Blackwell, Oxford.

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BIOLOGICAL TRAITS INCONSISTENT WITH PANGLOSSISM IN SPECIALIZED PHYTOPHAGOUS INSECTS

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"*& Pangloss* disait quelquefois à Candide: Tous les évènements sont enchaînés dans le meilleur des mondes possibles; car si vous n'aviez pas été chassé d'un beau château à grands coups de pied dans le derrière pour l'amour de mademoiselle *Cunégonde*, si vous n'aviez pas été mis à l'inquisition, si vous n'aviez pas couru l'Amérique à pied, si vous n'aviez pas donné un bon coup d'épée au baron, si vous n'aviez pas perdu tous vos moutons du bon pays d'Eldorado, vous ne mangeriez pas ici des cédrats confits & des pistaches." *Voltaire: Candide*, 1784, Oeuvres complétes, tome 44, p 342.

"La nature paraît avoir suivi les mêmes proportions dans les diverses grosseurs des fruits destinés à nourrir l'homme, que dans la grandeur des feuilles qui devaient lui donner de l'ombre dans les pays chauds; car elle y en a taillé pour abriter une seule personne, une famille entière, et tous les habitants d'un même hameau." *Jacques-Bernardin-Henri de Saint-Pierre: Etudes de la Nature*, t. 2, à Bâle, chez Tourneizen, 1797.

"Although beyond even the wildest of a Darwinist's dreams, it is just possible that some plants can evaluate the kind of leaf-clipping herbivore to which they have been subject and generate an appropriate facultative response" *D. H. Janzen*, 1979.

ABSTRACT

Plant-insect relationships are used to illustrate coevolution. It is difficult to understand independent plant and insects evolutions. Since Fraenkel, biochemical escalation explains coevolutive trophic relations.

To study coadaptative traits, it is absolutely necessary to choose the evolving habitat and biocenosis, which exclude any interpretation issued from the cultivated field or from the lab.

Protection of reproductive organs is a prerequisite only in annual plants, but many examples about the importance of chemical seed protection come from perennial tropical legumes. So, the actual situation is not so clear.

Bruchus affinis-Lathyrus sylvestris is a good model to elucidate these relations. We do not observe any plant adaptive traits in order to protect against bruchids'attacks; simultaneously, some traits of the insect biology are clearly adaptive, others are neutral, and some others are very disadvantageous with the point of view of the insect life history strategy.

Key words: coevolution, specialized insects, partial adaptation, reductionism, plasticity, agrosystem, habitat selection, contagious distribution, celery fly, bruchids, wild legumes, panglossism

INTRODUCTION

Like many entomologists working on phytophagous insects in the fifties, I was particularily disappointed with the use of quantitative differences of the plant basic compounds to explain host-plants relations. So, I was deeply stimulated by the reading in 1959 of the provocative G. Fraenkel's paper: "*The raison d'être of secondary plant substances*". Immediately, I adopted the coevolutive point of view and I diffused it in the French ecological community. I explained co-adaptation by a scheme of chemical arms race with coevolutive escalation. But, as "*adaptation is never the result of an isolated selective pressure,*" I considered "*each pressure is a part of the overall selective pressure of a certain environment.*" (Labeyrie, 1976).

Today, I maintain that evolution of living beings did not proceed from independent events and that coevolution is the corollary of trophic relations, but I am afraid of the abuse of coevolutive explanation to justify all the biological traits of the insect as well as the biological characteristics of the plant. Although, theoretically, for a short time scale, coevolution must stabilize both insect and plant numbers, curiously, the coevolutive explanation frequently drives to a reductionistic position in insect-plant studies. Separating biological activities between particular strategies is not considered only as an analytical mean to investigate more precisely every particular aspect, but rather as an atomistic approach proceeding from the ultra-deterministic process of genetic basis of behavioural traits. So, the discovery of plant allelochemical compounds strengthens the co-adaptive view by an atomistic explanation. For example, if the underlining of the chemical basis of insect-plant relationships is a truism, it is not right to amplify the influence of plant particular chemicals up to correlate each step of the colonization of the host by an insect with a particular plant product. The operational consequence of the principle: "one compound, one trophic relation", is the search for cultivars with higher allelochemical content.

To summarize a strict application of the coevolutive approach, specialized insects would be adapted to specialized compounds, and any change in the chemical content of the plant would modify the trophic relations. So, it should be sufficient to put into evidence some specific chemicals, and to exemplify it or to interchange one by another to obtain crop protection. The supporters of this concept hope that genetic engineering shall promote resistant plants by introducing genes coding adequate allelochemical compounds.

The two characteristics of the diehard coevolutive point of view equal the dual aspect of panglossism: angelolatry and ultra-determinism. Like Doctor Pangloss, many applied entomologists go on using this reductionist postulate and go on ignoring that Dethier (1976) underlined that "*differences among plants in the acceptable series were as great as between acceptable and unacceptable plants... The evidence suggests that the receptors are not rigidly specific. Each responds to a variety of compounds in a manner that is not constrained by chemical relationships.*" As a matter of fact, Raffa and Berryman (1982) observed that trees resistant to Scolytidae have no higher monoterpene levels, have no different relative proportions of the most toxic material, or at least more frequent occurrence of certain chemical groups. Raffa

(1986) notices "*mere identification of an active compound is not enough to promote crop protection*". In addition, the plant-breeder production of pest-resistant cultivars, increases "*amounts of natural pesticides. It should be no surprise, then, that a newly introduced variety of insect-resistant potato had to be withdrawn from the market, due to acute toxicity to humans caused by much higher levels of the teratogens solanine and chaconine than are normally present in potatoes.*" (Ames and Gold, 1989).

Many considerations about coevolution of plants and insects proceed from studies in fields, that are outside an evolutive context. Indeed, agrosystems differ fundamentally from ecosystems; they have a tendency to suppress any genetic continuity, since every year foreign isogenized seeds are introduced and the reproductive products of the system are removed. Simultaneously, the system is simplified and its periodicities aggravated. Its characteristics being more homogenous and more cyclic, it is clear that habitat relations of the insect species are drastically reduced in an agrosystem that leads to priviledge some specialized pests in cultivated plants exploitation. With such a reduced background and such a strengthened phenotypic homogeneity, it is possible to differentiate the aggressivity of different strains of insects or to note the susceptibility of different cultivars. Conversely, fluctuations of the population level are more frequent and higher in agrosystems, and the observer is less attempted to see an "equilibrium of Nature", in other words to commit Panglossism.

Consecutively, in applied entomology, panglossism is so frequent but less shown off than in conservation ecology.

BIOCENOTIC RELATIONS AND SOPHISTICATION OF THE SEXUAL BEHAVIOUR

Dobzhansky (1977) recalls the complexity of sexual behaviour in *Drosophila* due to the interlocking of many sensorial stimulations of different kinds. He underlined the particular complexity of the courtship behaviour of many Hawaiian species. Hawaiian *Drosophila* fauna is particularly rich. Now, "*courtship patterns may help in sexual isolation in that the movements and postures used may serve to display specific colours or scents in a conspicuous fashion. Sexual isolation can be defined as a behavioural barrier to hybridization between species or populations*" (Manning, 1979).

Perhaps the explanation on this sophistication of the courtship behaviour lies in a biocenotic concentration of related species induced by a common feeding place as it is observed for Tephritidae. Prokopy (1968) discovered that some trees played the role of *lieux de rendez-vous*. But, the *lieu de rendez-vous* is not necessarily the host-plant or the oviposition site, alternatively it can be the *restaurant* (Labeyrie, 1976). So, Leroi (1975) observed *Philophylla heraclei* mating inside the trees' canopy near the celery (*Sepium graveolens*) plants, the adults consuming honeydew of aphids or nectar of leaves. These restaurants, and at the same time mating places, are very cosmopolitan, and consecutively, sexual isolation in such an occupied lieu de rendez-vous implies sophisticated courtship behaviours (Labeyrie, 1978).

Two important aspects can be drawn from this observation. First, nature and complexity of the courtship are biocenotically related; there are no sexual co-adaptations alone, they also depend on the risk of confusion. Operationally, this implies that it is impossible to analyse and to understand sexual behaviour *in abstracto*, outside the ecological context of the population. Second, the trophic context is not limited to larval trophic relations, in holometabolous species at least, adult and larval niches differ frequently, and the biocenotic relations enlarge and include different categories of plants or even many organisms with different trophic levels. Indeed, the habitat used by a population necessarily includes more space and more diversity than assumed by larval feeding studies only. So, the combination of these complementary elements reduces the frequency of propitious situations. Thus, we have the opportunity to manage an uncomfortable agrosystem to protect it against pests.

INSECT ADAPTATION VERSUS COEVOLUTION

Acanthoscelides obtectus is an American bruchid, probably Colombian, diffused through the world with the commercial trade of *Phaseolus vulgaris*. Its colonization of many countries of the world from the equator to the latitudes of forties, is an exceptionally successful run (Labeyrie, 1989a). Its aptitude for taking advantage of stored seeds causes it to be a very dangerous cosmopolitan pest. Its gregarious egg-laying behaviour multiplies exponentially the efficiency of pioneer invaders.

The gregarious distribution of eggs into immature pods can be interpreted, like in the case of *Cactoblastis cactorum* on *Opuntia inermis* and *O. stricta*, as an elaborate strategy of the moth to maintain the larvae's resources (Monro, 1967). "*This behaviour, theoretically at least, should enhance stability*" (Caughle, 1976). As a matter of fact, even when there were very important attacks of *A. obtectus* in the fields, we never observed contamination of all the mature pods. As the density of *Opuntia* in Queensland was as high as that of the beans in the fields, it is very attractive to use the same explanation for the gregarious distribution of the bruchid, and to proclaim the deep wisdom of this insect the strategy of which serves to maintain its trophic basis.

Unfortunately, when we study the biocenotic relations of *A. obtectus* in its native country (altiplanos of Colombia), it becomes no more possible to exemplify the deep wisdom of this bruchid. In Columbia, wild bean is a perennial vine (we do not know the maximum age of each vine) climbing upon different bushes. The distribution of this plant is patchy and its fructification is very irregular. So, the presence of pods varies strongly, sometimes pods are rare. This insect not only shows a gregarious oviposition, but the overloading of the seeds with larvae also result in dwarfishness of adults. It is possible that the gregarious distribution of eggs and the ability to survive overexploitation of seeds by dwarfing, maintain populations with a sufficient gene pool when pods are rare in the field. If this is true, it is possible to consider adaptation of the insect without any advantage to the plant. Moreover, the mature

seeds seem without interest for this plant that is able to survive many years without sexual reproduction.

The only way to understand the actual biocenotic relations between *A. obtectus* and *P. vulgaris* was to go in their original habitat, where wild plants coexist with their consumers without human interference. Before concluding on co-evolutive strategies the minimal prerequisite is to observe the actual situation in the evolutive habitat.

The study of *A. obtectus* in Mexico revealed two sympatric annual cycles. Near Tepoxlan, *Acanthoscelides* attacks cultivated *P. vulgaris* and wild *P. coccineus*, but germination of cultivated *P. vulgaris* seeds is immediate, while germination of wild *P. coccineus* seeds is delayed. The bruchids bred on cultivated *P. vulgaris* do not enter diapause, while bruchids bred on wild *P. coccineus* seeds have diapause. The first explanation (Biemont and Bonet, 1981) was the polymorphic annual cycle of Tepozlan populations. But, a more cautious examination revealed the coexistence of two species of *Acanthoscelides*. The diapausing *A. obvelatus*, probably originating from Mexico with periodic conditions (annual arid season), has developed a cyclic activity like the plant. The non-diapausing *A. obtectus*, probably originating from equatorial altiplano of Latin America, has been introduced to Mexico by precolombian trade with cultivated beans. Today, both species live in sympatry in the Tepozlan area. So, there was no modification of the *A. obtectus* cycle to adjust to the phenology of Mexican wild *P. coccineus*.

AN AMBIGUOUS SITUATION: *LATHYRUS SYLVESTRIS* AND *BRUCHUS AFFINIS*

Maturing seeds of *L. sylvestris* are consumed by a univoltine bruchid, *B. affinis*. The seeds contain homoserine and α-γ-diaminobutyric acid (Rosenthal, 1981), and the presence of L-α-γ-diaminobutyric acid (DABA) makes *L. sylvestris* the most toxic *Lathyrus* for the mammals (Roy et al., 1986). *B. affinis* is known only to develop in maturing seeds of 3 species of *Lathyrus*. So, we observe apparently a typical coevolutive example. The plant develops allelochemical compounds against consumers to protect its reproductive organs; and all the seminivorous insects are excluded except *B. affinis* that elaborates some enzymes able to hydrolyse the toxic compounds. *L. sylvestris* would survive because *B. affinis* would not destroy alone sufficient number of seeds to compromise plant survival, and *B. affinis* would maintain itself because the costs of allelochemical compounds production and the loss of time and energy used to search for their host would be lower than the advantage offered by an empty niche.

Precise examination of the biocenotic relations between the plant and its bruchid pest shows, however, a more complicated situation. I have described some aspects of it in previous papers (Labeyrie and Hossaert, 1985; Labeyrie, 1989b). Below I summarize and add some points.

Is the bruchid very dangerous to L. sylvestris ? On the one hand, *B. affinis* gives some benefit to the plant by pollinating many flowers. It is possible that its contribu-

tion is significant and modifies the reproductive success of the plant (Hossaert and Valero, 1986). Pollination occurs when the adult moves into the flowers to eat pollen, it makes stigmate receptive by tickling, and carries auto- and allopollen. It seems that the adult does not look for nectar during its stay inside the flower; reward is direct since consumption of pollen is the prerequisite of oogenesis (Bashar et al., 1987).

But, on the other hand, fundamentally, it is possible that the loss of seeds due to the bruchids is without importance for these perennial plants with vegetative propagation. It is possible that the sexual reproduction is a relict of a previous time, when this plant survived only by sexual reproduction. Today, *L. sylvestris* lives with a tremendous low productivity of its reproductive organs (Hossaert and Valero, 1989), its flower is a luxury (maybe to amuse romantic strollers!). More seriously, seed production appears only as an insurance against ecological hazards, and it is possible that climatic events destroying rhizomatic network would induce germination of seeds of soil bank (Hossaert and Gauthier, 1980).

Thus, it appears that it is impossible to conclude that *B. affinis* today is pernicious to *L. sylvestris.*

Is the exploitation of an empty niche advantageous to B. affinis? Though *B. affinis* is the only insect issued from mature seeds of *L. sylvestris*, some other insects attack maturing seeds in the pods. These attacks by other insects are detrimental to bruchid larvae; indeed, *Laspeyresia* caterpillars or *Tychius* larvae eating young seeds destroy and consume at the same time the bruchid larvae growing in the seed. The occurrence of DABA, highly toxic to mammals, must exclude rodents from *L. sylvestris* consumers, but *Apodemus sylvaticus* (P. Haffne, personnal communication) eats mature seeds and particularly the ones with bruchids in their cotyledons. They decorticate the tegument to have access to cotyledons, and they decorticate cotyledons to have access to the bruchids. To collect seeds of *L. sylvestris* before dehiscence of pods *A. sylvaticus* climbs on the bushes to cut pods; after the dehiscence, it gathers the seeds upon the soil and it consumes the last adults even inside the seeds.

So, even in a relatively simple system, as a pod, trophic relations are not very simple, and some plant consumers act as the insects' predators. Consequently, it is not sufficient to record seminivorous insects and to examine what species can emerge from collected seeds, to conclude about interspecific competition. Even strict specificity of this category of consumers does not exclude intraspecific competition, or more exactly, interspecific interlocking.

Does a specialized niche prevent resource limitation? B. affinis is very finely specialized, it lays its eggs upon only three species of *Lathyrus*, consequently, after the abscission of flowers, there is generally a sufficient number of young pods to maintain the level of population of this bruchid. Theoretically, the conditions are the best ones to limit or to suppress intraspecific competition. Concurrently, attacks of other consumers of pods are confined to restricted places and periods. It seems *a priori* that interspecific competition is not a general problem, even if it introduces hazards during the larva's growth.

Actually, the offspring wastage before the definitive establishment of the larvae into the seed is tremendous. We enumerate four different reasons of mortality (N'Diaye and Labeyrie, 1989), but the most important one is that the distribution of eggs between pods is not adjusted to the seeds available in the pods. We observed up to more than 100 eggs on a pod containing only 7 or 8 seeds while only one larva develops in one seed! At the same time, other pods receive no eggs.

Direct observation of the foraging behaviour explains this aggregative distribution of the eggs. After landing on the attractive flowers of *L. sylvestris*, adults explore the bush apparently at random, walking on the stems or the leaf stalks of the different plants interlocking into the bush superficial structures. They do not seem to be attracted by the pods. But, when they discover a pod, they immediately prospect it carefully. But, pods of different inflorescences are interlocked; consecutively, they prospect successively all the lot of interlocked pods. The patchy distribution of the pods results in aggregative distribution of eggs upon some pods on the bush. Females lay their eggs at random on the pod without any consideration of the place of aborted or fertilized ovule.

The direct observation of the oviposition behaviour refutes anthropomorphic assumptions on the strategy of the bruchid, namely, that similar to *Cactoblastis*, it is able to save one part of its plant capital for the future of its offspring!

In fact adaptation of *B. affinis* to *L. sylvestris* is not perfect, and it is not necessary to refer to Doctor Pangloss to describe distribution.

CONCLUSIONS

One of the most contradictory aspects of the study of concrete biocenotic situations is the occurrence of some specific adaptive traits together with others the adaptive value of which seems questionable. Such is particularly the case of *B. affinis* (Labeyrie, 1989b). If adaptation of this specialized consumer is partial, it is impossible to discover in *L. sylvestris* host traits that are influenced by the activity of the insect.

It is yet difficult to avoid panglossism when we analyse the biological traits and their possible adaptive value (Gould and Lewontin, 1979). Behaviour is the result of diverse information received from the habitat and from some particular parts of the host and it is modulated by previous experience and by the physiological state of the insect. So it is impossible to claim that a particular answer is always induced by the same stimulus. It is a general feature of adaptation that each organism, with its own genetic information and with its own history is adapted to particular situations, i.e. to complex entities of factors. To search for a one for one relation necessarily drives to panglossism.

If it is not easy to establish the actuality of an adaptationist programme of a specialist insect, it is several times more difficult to discover coevolutive relations. Jermy (1976) put five questions that he considers as prerequisites to coevolutive

explanation. I think he is right, even if coevolutive consequences cannot be restricted to production of allelochemical compounds by the plant.

Predation pressure can also modify some circadian or annual aspects of the life cycle as well as some morphological features as it is supposed with pollination biology, even, if, curiously, the term of coevolution is not used by Free in 1970, in "Insect pollination of crops". Maybe, it is significant that, to introduce the coevolutive approach in pollination, Bergstrom (1987) chooses to cite a vulgarization book about "la vie des plantes". But this choice does not mean that coevolutive explanation was not more seriously used to explain coevolution.

In the second French edition of "L'Origine des Espèces", Darwin wrote (chapter IV): "*Les insectes anthophiles, et les plantes dont ils se nourrissent, exercent les uns sur les autres une action réciproque qui peut être plus ou moins puissante... On voit ainsi comment une fleur et un insecte peuvent simultanément ou l'un après l'autre se modifier et s'adapter mutuellement de la manière la plus parfaite, au moyen de la conservation continue d'individus présentant des déviations de structures particulières et réciproquement avantageuses*". It is true that in the next lines Darwin emphasized that his examples should produce objections; but he refused them scornfully, because previous objections to geomorphologism of Ch. Lyell, were wrong.

It is sure that the production by *Ophrys* flowers of chemical substances that mimic "*insect volatiles which might well earlier have been food signals but which are now part of the species recognition signal*" (Bergstrom, 1987) is difficult to imagine without a coevolutive point of view.

If the coevolutive explanation is the consequence of the occurence of particular, strict, reciprocally advantagous relations, and if it is difficult to imagine an other explanation, it is incorrect to extend the coevolutive paradigm to all the aspects of trophic relations.

The first question is: How important is the selective pressure induced by the consumer?

To know this importance, it is necessary to consider the whole life history of the host and not only to observe the loss upon one organ at a particular time.

In conclusion of the collective book about coevolution Futuyama and Slatkin (1983) remark: "*Almost all the authors in this volume have emphasized that diffuse coevolution is likely to be far more common than pairwise coevolution, but models of diffuse coevolution have hardly been developed. The models will no doubt be complex; but it may be the very complexity of the interactions among species that accounts for the variable pace of coevolution.*" In their study about "Coevolution and the fossil record", Stanley et al. (1983) conclude: "*We suggest that coevolution is not, in general, the precise, effective, and persistent phenomenon that it has often been portrayed to be*".

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REFERENCES

Ames, B. N. and L. S. Gold (1989): Pesticide, risk, and applesauce. *Science 244,* 755-757.

Bashar, A., G. Fabres, M. Valero and V. Labeyrie (1987): *Bruchus affinis* and the flowers of *Lathyrus latifolius*: an example of the complexity of relations between plants and phytophagous insects. In: V. Labeyrie, G. Fabres and C. Lachaise (eds), *Insects-Plants*. Junk, Dordrecht. 189-194.

Biemont, J. C. and A. Bonet (1981): The bean weevil populations from the *Acanthoscelides obtectus* Say group living on wild or subspontaneous *Phaseolus vulgaris* L. and *P. coccineus* L. and on *P. vulgaris* cultivated in the Tepozlan region Morelos) Mexico. In: V. Labeyrie (ed), *The Ecology of Bruchids Attacking Legumes (Pulses)*. Junk, Dordrecht. 23-41.

Bergstrom, G. (1987): 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? In: V. Labeyrie, G. Fabres and D. Lachaise (eds), *Insects-Plants*. Junk, Dordrecht. 321-328.

Caughley, G. (1976): Plant-herbivore system. In: R. M. May (ed), *Theoretical Ecology, Principles and Applications.* Blackwell, Oxford. 94-113.

Dethier, V. (1976): The importance of stimulus patterns for host-plant recognition and acceptance. In: T. Jermy (ed), *The Host-Plant in Relation to Insect Behaviour and Reproduction*. Plenum Press, New York. 67-70.

Dobzhansky, Th. (1977): Mécanismes éthologiques de l'évolution: synthèse. In: J. Médioni and E. Boesiger (eds), *Mécanismes Éthologiques de l'Évolution*. Masson, Paris. 142-143.

Fraenkel, G. S. (1959): The raison d'être of secondary plant substances. *Science 129,* 1466-1470.

Free, J. B. (1970): *Insect Pollination of Crops.* Acad. Press, London.

Futuyma, D. J. and M. Slatkin (1983): The study of coevolution. In: D. J. Futuyma and M. Slatkin (eds), *Coevolution.* Sinauer, Sunderland. 459-464.

Gould, S. J. and R. C. Lewontin (1979): The sprandels of San Marco and the panglossian paradigm: a critique of the adaptationist programme. *Proc. Roy. Soc. London, B 205,* 581-598.

Hossaert-Palauqui, M. and N. Gauthier (1980): Régénération d'une lande après incendie: 2 reproduction sexuée et capacité de germination d'*Ulex minor* R. *Bull ecol., 11,* 387-392.

Hossaert, M. and K. Valero (1986): Vegetative propagation and sexual reproduction in 2 perennial *Lathyrus* species. In: A. K. Kaul and D. Combes (eds), *Lathyrus and Lathyrism*. TWMRF Publ., New York. 175-183.

Hossaert, M. and K. Valero (1989): Effect of ovule position in the pod on the patterns of seed formation in 2 species of *Lathyrus*. *Amer. J. Bot. 75,* 1714-1731.

Janzen, D. H. (1979): New horizons in the biology of plant defense. In: G. A. Rosenthal and D. H. Janzen (eds), *Herbivores, Their Interaction with Secondary Plant Metabolites*. Acad. Press, London. 331-350.

Jermy, T. (1976): Insect-host-plant relationship— coevolution or sequential evolution? In: T. Jermy (ed), *The Host-Plant in Relation to Insect Behaviour and Reproduction*. Plenum Press, New York. 109-114.

Labeyrie, V. (1976): The importance of the coevolutive point of view in the investigation of the reproductive relations between insects and host-plants. In: T. Jermy (ed.), *The Host-Plant in Relation to Insect Behaviour and Reproduction*. Plenum Press, New York. 133-137.

Labeyrie, V. (1977): Environnement sensoriel et coévolution des insectes. In: V. Labeyrie (ed), *Comportement des Insectes et Milieu Trophique*. CNRS, Paris. 15-35.

Labeyrie, V. (1978): Reproduction of insects and coevolution of insects and plants. *Entomol. exp. appl. 24,* 496-499.

Labeyrie, V. (1989a): The bean beetle, *Acanthoscelides obtectus* and its host, the French bean, *Phaseolus vulgaris*; a 2 ways colonization story. In: F. di Castri and A. J. Hansen (eds), *Biological Invasions in Europe and the Méditerranéan Basin*. Junk, Dordrecht. (in press)

Labeyrie, V. (1989b): Is it sufficient to consider plant-insect relationship to conclude about coevolution? In: J. Maynard-Smith and G. Vida (eds), *Organisational Constraints on the Dynamics of Evolution*. Manchester Un. Press, Manchester. 273-283.

Labeyrie, V. and M. Hossaert (1985): Ambiguous relationships between *Bruchus affinis* and the *Lathyrus* group. *Oikos 44,* 107-113.

Leroi, B. (1975): Importance des arbres pour les populations d'adultes de la mouche du céleri, *Philophylla heraclei* L. (Dipt. Tephrit.). *C. R. Acad. Sc. Paris, 281D,* 289-292.

Manning, A. (1979): *An Introduction to Animal Behaviour*. Arnold, London. 329 p.

Monro, J. (1967): The exploitation and conservation of resources by populations of insects. *J. anim. Ecol. 36,* 531-547.

N'Diaye, S. and V. Labeyrie (1989): Etude de l'adaptation de *Bruchus affinis* à *Lathyrus silvestris*: analyse de la mortalité avant l'installation des larves dans les graines. *Entomol. exp. appl.* (submitted)

Prokopy, R. J. (1968): Visual responses of apple maggot flies *Rhagoletis pomonella* (Dipt., Tephrit.): orchards studies. *Entomol. exp. appl. 11,* 403-422.

Raffa, K. F. (1986): Devising pest management tactics based on plant defense mechanisms, theoretical and practical considerations. In: B. Brattsten and S. Ahmad (eds), *Molecular Aspects of Insect-Plant Associations*. Plenum Press, New York. 320-327.

Raffa, K. F. and A. A. Berryman (1982): Physiological differences between lodgepole pines resistant and susceptible to the mountain pine beetle and associated micro-organisms. *Environ. Entomol. 11,* 486-492.

Rosenthal, G. A. (1981): Role of the allelochemics in the specialisation of trophic relations between bruchids and legumes. In: V. Labeyrie (ed), *The Ecology of Bruchids Attacking Legumes (Pulses)*. Junk, Dordrecht. 97-100.

Roy, D. N., P. S. Spencer and P. B. Nunn (1986): Toxic components of *Lathyrus* In: A. K. Kaul and C. Combes (eds), *Lathyrus and Lathyrism*. TWMRF Publ., New York. 287-296.

Stanley, S. M., B. Van Valkenburgh and R. S. Stenck (1983): Coevolution and the fossil record. In: D. J. Futuyama and M. Slatkin (eds), *Coevolution*. Sinauer, Sunderland. 328-349.

EVOLUTION

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PHYLOGENY AND HOST PLANT SPECIALIZATION: SMALL ERMINE MOTHS (*YPONOMEUTA*) AS AN EXAMPLE

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ABSTRACT

Since 1971 small ermine moths have been studied in many different ways. All these approaches, however, were aimed at the relation between the insects and their host plants. Behavioural and sensory reactions of the larvae towards relevant plant constituents were assessed. In these studies an answer was sought on the question of the host plant specificity of a number of *Yponomeuta*-species.
Along with the elucidation of the chemical composition of the sex pheromones the sensitivity and morphology of the sense organs on the antennae of males and females was investigated. Attempts were undertaken to detect links between courtship behaviour and oviposition, i.e. host plant selection.
In separate studies much attention has been devoted to numerical and biochemical taxonomy. A selection of the findings will be discussed and where possible integrated.

Key words: taxonomy (numerical, biochemical), reproductive isolation, pheromones, host plant selection, specificity, host shift, life history

INTRODUCTION

Small ermine moths of the genus *Yponomeuta* are characterized by fairly strict preferences for specific food plants. One of the eleven species intensively studied during the last decade, however, appeared to have less strict host ties, as it occurs with several, related, plant hosts. This led to the speculation that so-called host races might occur, representing the first step leading to new, separate species. Another intriguing aspect of the genus is the disagreement among taxonomists as to the number of species. Friese (1960), laying much weight on differences in genitalia, combined five species into one: the *padellus*-complex. Furthermore, the apple ermine moth — *Y. malinellus* — was, and often still is, referred to as a subspecies of *Y. padellus*.

As morphological similarities might reflect a relatively recent date of origin and the behaviour of host races might reveal aspects of speciation, the genus seems to be a very suitable model for a study on (micro) evolution.

From the beginning of our attempts to reconstruct the phylogeny of the genus, a multi(sub)disciplinary approach was chosen. Besides taxonomy, numerical as well as biochemical, several features of the reproductive isolation among the species were

Table 1 Small ermine moths and their food plants. Between () are the abbreviations used in the figures. See also Fig. 1.

Group A	*Y. malinellus* (mal)	*Malus sylvestris*
	Y. padellus (pad)	*Prunus spinosa, Crataegus* spp., *P. domestica, Sorbus aucuparia, Amelanchier lamarckii*
	Y. rorellus (ror)	*Salix* spp.
	Y. evonymellus (evon)	*Prunus padus*
	Y. cagnagellus (cag)	*Euonymus europaeus*
	Y. mahalebellus (mah)	*Prunus mahaleb*
	Y. irrorellus (irror)	*Euonymus europaeus*
Group B	*Y. plumbellus* (plum)	*Euonymus europaeus*
	Y. vigintipunctatus (vig)	*Sedum telephium*
Not depicted in Fig. 1	*Y. gigas* (gig)	*Salix canariensis, Populus alba*, "connected" with *Y. rorellus*
	Y. yanagawanus (yan)	*Euonymus japonica* (from Japan), belongs to group B.

investigated. Thirdly, food plant selection and acceptance, the chemical composition of the plants, the sensory reactions towards plant constituents by the larvae and the suitability for their normal development was investigated in much detail.

The present paper is the first trial to integrate a selection of the data obtained so far.

THE SPECIES AND THEIR HOST PLANTS

In Table 1 and Fig. 1 the names of the species and those of their host plants can be found. The phylogenetic tree used in Fig. 1 will be discussed below.

LIFE HISTORY DATA

Apart from the specificity of their food plants many traits in the biology of the small ermine moths are very similar. All but one have one generation a year, see also Fig. 2, and all occur in the same type of vegetation and during the same time of the year as larva and adult. *Y. vigintipunctatus* is the only species feeding on a herb instead of on shrubs or trees. *Y. plumbellus*, however, is also found in the herb-layer (on shoots of the creeping roots of its host plant). These members of group B also differ in hibernation, *viz.:* in the pupal or the egg- stage, respectively. Those of group A hibernate as first instar larvae under a common eggshield. *Y. irrorellus* being the exception as it starts boring into the twig of its host before entering diapause. In spring feeding activity begins as soon as it gets warm enough. There is, however, a constant variation among the species in the dates of "emergence". Due to the rela-

tively long life span of the moths encounters between males and females of the wrong species are certainly possible. Hybridization is also not avoided by calling at separate hours during the night. All members of species-group A call before or at dawn, those of group B around midnight, Hendrikse (1979).

A study of the parasitoid complexes and the patterns of parasitization showed characteristic differences among the species, Dijkerman (1987). Further they differ in the capacity to encapsulate eggs of the in some species important parasitic wasp *Diadegma armillata*. The distribution of this capacity among the species bears a relationship to the phylogenetic tree shown in Fig. 1 (Dijkerman, 1990).

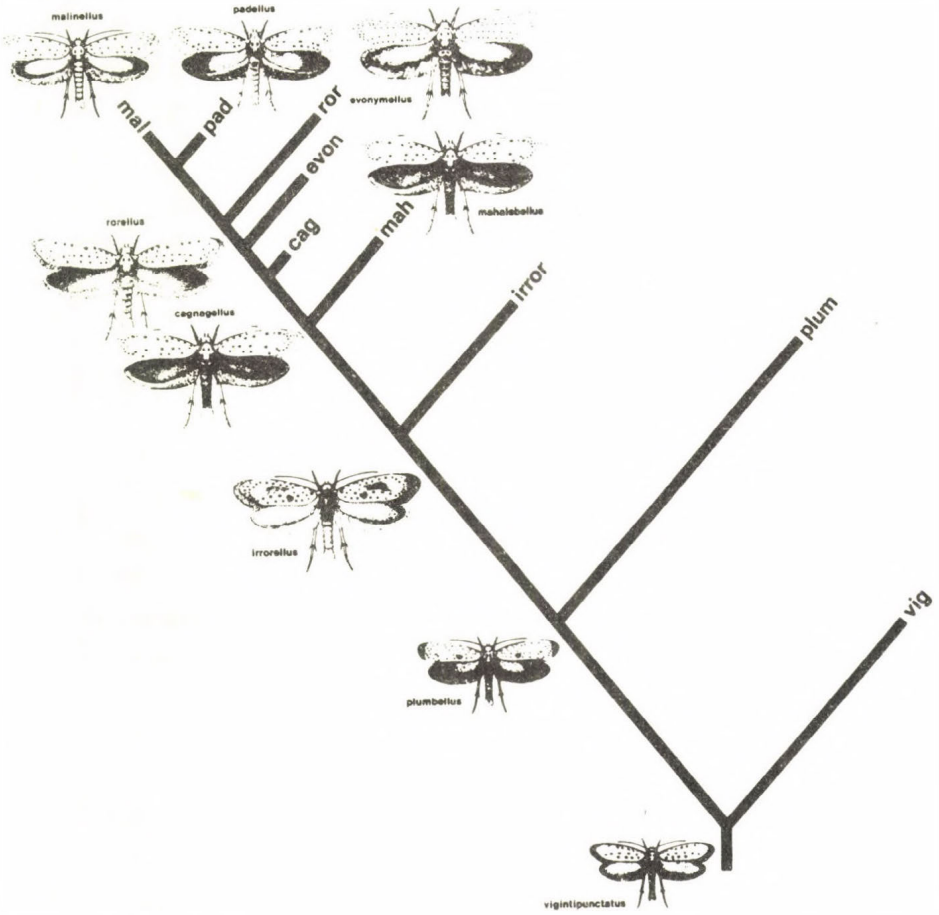

Fig. 1 Phylogenetic tree calculated from the original allozyme data of Menken (1982). The total length of the tree is 2.068 with 1.172 as the longest path (vig-ror). See Table 1. Adapted from Löfstedt et al. (1989).

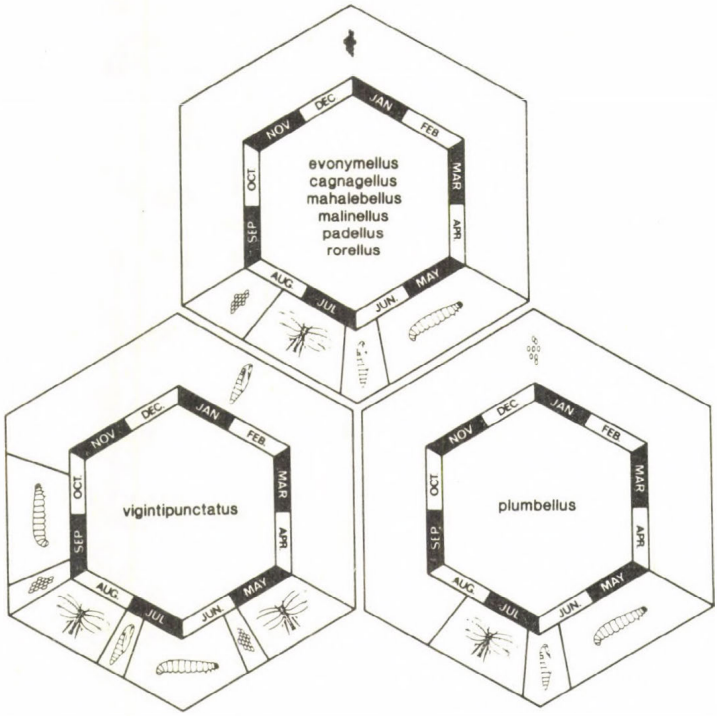

Fig. 2 Life cycles of eight *Yponomeuta*-species.

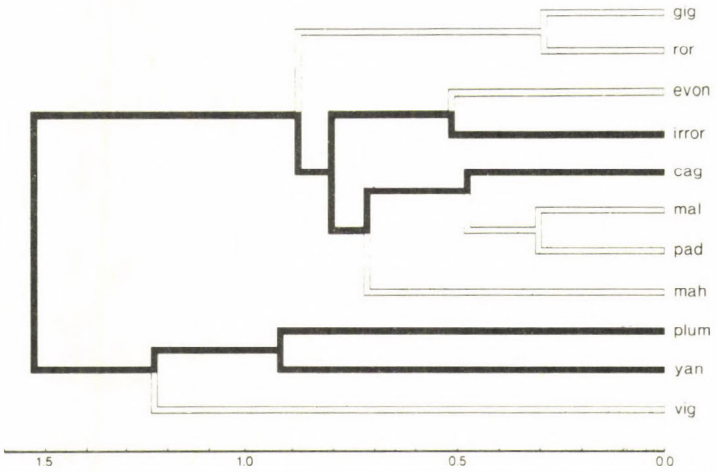

Fig. 3 UPGMA-dendrogram indicating dissimilarities in morphology among eleven species of *Yponomeuta*. Courtesy Dr. G. D. E. Povel.

NUMERICAL TAXONOMY

Using a vast amount of only morphological characters Povel (1986, 1987a, b) could demonstrate that specimens taken as larvae from their food plants and reared through emergence as moths, invariably felt into separate clusters, which corresponded well with the species-names already available. The separation of *Y. malinellus* and *Y. padellus,* however, was less perfect (see Povel 1987a, Fig. 9.10 on p. 376).

Povel used a number of approaches to calculate the relationships among the eleven species. A species-complex around *Y. padellus* did not emerge. For the purpose of this paper Povel produced the dendrogram shown in Fig. 3. To obtain a high predictability it is based upon the average of six dissimilarity-matrices, *viz.* as continuous characters: 111 shape and size-differences of 25 females and 130 of 25 males, plus two sets of binary characters, one matrix based upon the dots on the forewings and one on differences in genitalia (females and males combined in this instance). Fig. 3 represents the clustering (UPGMA) of this average dissimilarity.

BIOCHEMICAL TAXONOMY

In his search for the genetic relationships among the species of small ermine moths Menken (1982) applied the so-called zymogram method. An approach that has proven to be a powerful tool for investigating systematic and evolutionary problems.

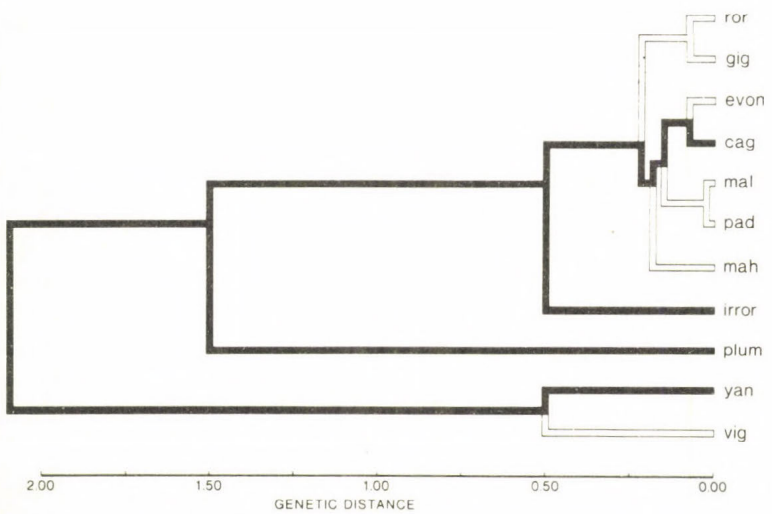

Fig. 4 Dendrogram for eleven species of *Yponomeuta* on the basis of 27 loci (adapted from Herrebout et al., 1982). *Y. gigas* and *Y. rorellus* have a smaller genetic distance than depicted here (Menken, pers. comm.).

The method consists of electrophoretic separation of enzymes and their subsequent specific staining. Here again all material studied was taken from their food plants and reared through till emergence. As a rule the samples originated from the same localities as those examined by Povel. The separate species could easily be told apart, again with the exception of *Y. malinellus* and *Y. padellus*, see, however, also Arduinno and Bullini (1985). A species-complex could not be detected; the distinct *Y. evonymellus* appeared to have a small genetic distance to the members of Friese's *padellus*-complex.

Fig. 4 is obtained by adding *Y. gigas* to the dendrogram shown in Herrebout et al. (1982), Fig. 5. The genetic distance (D) between *Y. gigas* and *Y. rorellus* is 0.065 (Menken, pers. comm.).

From the original 1982-data Menken and Ellis constructed the phylogenetic tree depicted in Fig. 1 using *Y. vigintipunctatus* as the outgroup. Further details are given by Löfstedt et al. (1989).

REPRODUCTIVE ISOLATION

A variety of features connected with courtship behaviour has been studied, several of them in detail. In six of the nine species presented in Fig. 1 the females produce a sex pheromone consisting of a specific blend of tetradecyl acetate and the isomers

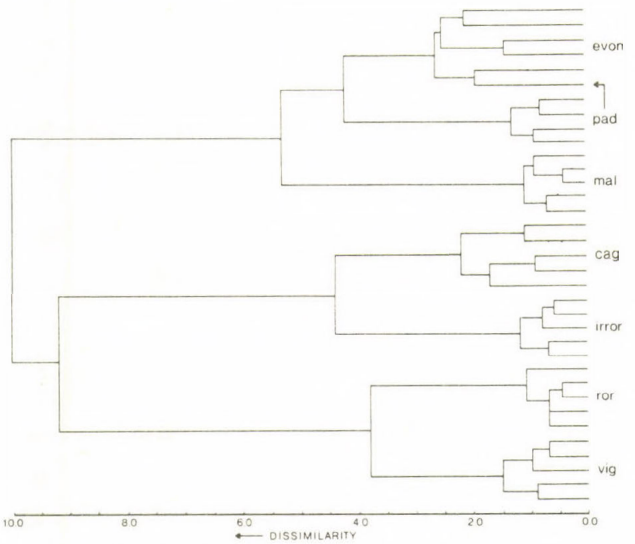

Fig. 5 Dendrogram of quantitative chemosensitivity patterns of seven species of *Yponomeuta* five specimens each. Compounds tested electrophysiologically were: sucrose (5), NaCl and KCl (3), sorbitol (3), dulcitol (3), phloridzin (3) and prunasin (1). Between () number of concentrations used. In one case only (*Y. padellus*) an exemplar felt into the cluster of another species. After Fig. 1 in Van Drongelen and Povel (1980).

E-11- and *Z*-11-tetradecenyl acetate. The three remaining species differ markedly from this situation. In *Y. malinellus*, in which the true nature of the pheromone is still not fully understood, acetate is replaced by alcohol. In *Y. mahalebellus* *Z*-11-hexadecenyl acetate is the major component. This compound is also part of the mixture made by *Y. padellus*, one of the six species referred to above. Finally, *Y. rorellus* stands out, even among all other Lepidoptera of which the pheromone composition is established. It uses not only one single compound, but it is also a saturated one (again tetradecyl acetate), Löfstedt et al. (1986). Interestingly enough this unique situation holds also for *Y. gigas* (Löfstedt, pers. comm.), a species endemic to the Canary Islands, where *Y. rorellus* does not occur. Two species – *Y. evonymellus* and *Y. vigintipunctatus* – produce an identical pheromone. In trap catches as well as in flight tunnel experiments cross attraction has been observed, Hendrikse (1986). In nature, however, the two species (genetically far apart) will hardly if ever mate. There are differences in seasonal appearance, see Fig. 2, and courtship takes place mainly at different heights in the vegetation, Herrebout and Van de Water (1983) and at separate hours during the night, Herrebout et al. (1984).

Females of some species postpone pheromone production for some time in the absence of the right host plant, Herrebout and Van de Water (1982), Hendrikse and Vos-Bünnemeyer (1987). Furthermore, they differ in their sensitivity to plant derived odours, Van der Pers (1981). This leaves room for the assumption that host plant selection preceeds calling activity (followed by oviposition). Sympatric speciation by host plant shift calls for a situation like this. A tendency to be attracted to their specific food plants in the first place before mating happens heightens the probability that indeed only the conspecific sexes meet each other. As females mate only once or twice there is always a surplus of males around, De Jong et al. (1988). Small ermine moths are always seen in aggregations. Therefore, a calling female will almost immediately be found after the start of pheromone release by their conspific male only. In combination with the efforts to detect the chemical composition of the volatiles synthesized in the pheromone glands, the corresponding odour perception by the males was also studied thoroughly, Van der Pers et al. (1980), Van der Pers (1982) and Cuperus (1986). Males appeared not only to be sensitive to compounds produced by conspecific females, but also to those released by the females of close relatives. For instance, males of *Y. padellus* showed a sensory (and a negative behavioural) reaction towards the alcohol emitted by *Y. malinellus*. The males of *Y. rorellus*, the species with the extremely reduced pheromone, avoided traps in which *Z*-11-tetradecenyl acetate was added to tetradecyl acetate. They also reacted electrophysiologically to the major compounds found in *Y. malinellus, Y. padellus* and *Y. mahalebellus*, Löfstedt et. al. (1989a).

The absence of hybrids among the specimens analyzed electrophoretically by Menken (1982) shows that the reproductive isolation is very effective in the field.

HOST-PLANT SELECTION

Unfortunately enough oviposition behaviour has received only occasional attention. Several attempts met with ambiguous results, Van de Water (1983), Herrebout et al. (1987), Kooi et al. (1989). The selection of a "wrong" host plant by the ovipositing female will only lead then to a shift towards a new host plant species when the larvae can cope with the new food. Therefore, the ability of larvae to complete their development on other plants than those of origin was tested thoroughly, Kooi (1990, this volume).

As a rule larvae clearly discriminate between hosts and non-hosts. In a few instances, however, the specific food plants could be interchanged, *viz.*: *Y. evonymellus* larvae developed normally on *Crataegus* (remarkably enough not on *Prunus spinosa*) and vice versa *Y. padellus* larvae on *Prunus padus*. As in the field such exchanges never seem to occur, it is obvious that the females make the right choices before laying their eggs.

As many of the plant species on which small ermine moths live grow very often side by side, host plant specificity, maintained by adults as well as by larvae (for instance migrating after defoliation) forms an essential element in the biology of the species.

A nice illustration of one of the aspects of it is shown in Fig. 5. Originally it was thought that indeed the sugar alcohol dulcitol did not occur outside the Celastraceae and that a dulcitol or sorbitol receptor in taste hairs of the larvae meant that the host plant of the species was *Euonymus* or one of the Rosaceae, respectively. Analysing the food plants of small ermine moths with methods more sensitive than those applied in the past, it was detected that dulcitol is not restricted to Celastraceae, but occurs also in *P. padus, P. spinosa* and probably in very low amounts in *P. mahaleb*, Fung and Herrebout (1988). The corresponding species of *Yponomeuta* living on Rosaceae are also sensitive to dulcitol. Peterson et al. (1989) detected that the electrophysiological reaction of *Y. evonymellus* is stronger to dulcitol than to the "normal" sugar alcohol sorbitol and further that at a low dose the spike frequency was 1.5 times higher than at a 40 times higher dose. In *Y. cagnagellus* the spike frequencies at the three doses tested were equal.

Attempts to establish differences among the so-called host races of *Y. padellus* did not reveal consistent patterns in oviposition preference by the adults or food preferences or their suitability as such for the larvae.

PHYLOGENY

Comparison of the dendrograms in which the black lines lead to *Euonymus* spp. as given in Fig. 3 and 4 and other approaches to express the relationships among the *Yponomeuta*-species, Povel (1987a, b), creates a rather consistent image. This might reflect the phylogeny of the genus already. The only phylogenetic tree calculated so far is presented in Fig. 1, which, as is mentioned already before, is based on

Menken's (1982) biochemical data. A comparable tree based upon the morphological data will be calculated in the near future. Povel (pers. comm.) also demonstrated that a much clearer picture of the history of the genus is obtained when non-European members are included as well. For the time being the tree shown in Fig. 6 using all the information available is seen as a fair summary.

Note that *Y. evonymellus* is no longer treated as belonging to a group around *Y. polystigmellus*, Gerrits-Heybroek et al. (1978). The two species with the same "abnormal" host (*Salix* spp.) have a very small genetic distance indeed and are very similar in morphology, Povel and Herrebout (1986). As *Y. gigas* was not studied in as much detail as *Y. rorellus* the species could not be included in the calculations for the phylogenetic tree in Fig. 1 (the same applies for *Y. yanagawanus*). Another interesting pair form *Y. malinellus* and *Y. padellus*, genetically and morphologically they are deceptively similar. They differ, however, strikingly in very significant biological features. The pheromone produced by females of *Y. malinellus* repelling the males of *Y. padellus* seem to demonstrate a reproductive isolating mechanism in action. The loss in taste sensitivity to phloridzin enables the larvae of the apple ermine moth to exploit a food source not available to all other *Yponomeuta*-species.

The double taste sensitivity of the larvae of *Y. evonymellus, Y. padellus* and *Y. mahalebellus* for sorbitol and dulcitol, enigmatic at first, Van Drongelen (1979), is easily explained by the detection of low amounts of this sugar alcohol in the *Prunus* species upon which these small ermine moths feed. The greater sensitivity of the larvae of *Y. evonymellus* to dulcitol than to sorbitol is highly remarkable in this respect. It is tempting to speculate upon the hypothesis that the presence of dulcitol in some rosaceous plants has facilitated the shift from a celastraceous lineage to

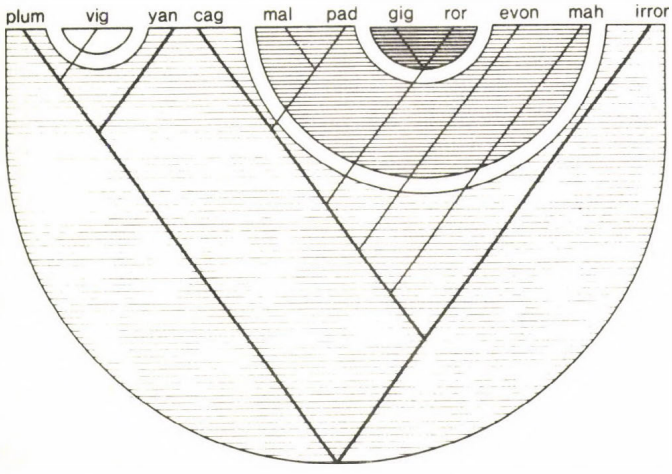

Fig. 6 Schematic representation of the phylogeny among eleven species of *Yponomeuta*. The largest surface comprises species on Celastraceae, the smallest the one on Crassulaceae, the remaining two areas the four species on Rosaceae and the two on Salicaceae. For abbreviations see Table

Prunus species as hosts. If this is the case the ancestor of *Y. malinellus* and *Y. padellus* may have made the first step to *Prunus spinosa,* followed later by a shift of *Y. malinellus* to apple and *Y. padellus* to *Crataegus* (remaining, however, on *P. spinosa* as well). *Y. padellus* also thriving on *Prunus domestica, P. cerasifera, Sorbus aucuparia* and *Amelanchier lamarckii*, still remains a good candidate for research on sympatric speciation through host shifts.

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REFERENCES

Arduino, P. and L. Bullini (1985): Reproductive isolation and genetic divergence between the small ermine moths *Yponomeuta padellus* and *Y. malinellus*. *Atti Acc. Lincei Mem. fis. S. VIII, Vol. XVIII, Sez. III fasc. 2*, 32-61.

Cuperus, P. L. (1986): A comparative electron microscopical study on antennae of small ermine moths (Lepidoptera, Yponomeutidae). *Thesis University of Groningen, The Netherlands.* 144 pp.

DeJong, M. C. M., K. Veenstra and W. M. Herrebout (1988): Asymmetry in potential for sexual selection. Chapter 7 (:115-131) In: M. C. M. DeJong, Evolutionary approaches to insect communication systems. *Thesis University of Leiden, The Netherlands*. 163 pp.

Drongelen, W. van (1979): Contact chemoreception of host-plant specific chemicals in larvae of *Yponomeuta* species. *J. Comp. Physiol. 134,* 265-279.

Drongelen, W. van and G. D. E. Povel (1980): Gustatory sensitivity and taxonomic relationships in larvae of some *Yponomeuta* species. *Proc. Kon. Ned. Akad. Wet. (C) 83,* 121-125.

Dijkerman, H. J. (1987): Parasitoid complexes and patterns of parasitization in the genus *Yponomeuta* Latreille (Lepidoptera, Yponomeutidae). *J. appl. Entomol. 104,* 390-402.

Dijkerman, H. J. (1990): Suitability of eight *Yponomeuta* species as hosts for *Diadegma armillata*. *Entomol. exp. appl. 54*, 173-180.

Friese, G. (1960): Revision der paläarktischen Yponomeutidae unter besonderer Berücksichtigung der Genitalien. *Beitr. Entomol. 10,* 1-131.

Fung, S. Y. and W. M. Herrebout (1988): Sorbitol and dulcitol in some Celastraceous and Rosaceous plants, hosts of *Yponomeuta* spp. *Biochem. Syst. Ecol. 16,* 191-194.

Gerrits-Heybroek, E. M., W. M. Herrebout, S. A. Ulenberg and J. T. Wiebes (1978): Host plant preferences of five species of small ermine moths. *Entomol. exp. appl. 24,* 360-368.

Hendrikse, A. (1979): Activity patterns and sex pheromone specificity as isolating mechanisms in eight species of *Yponomeuta. Entomol. exp. appl. 25,* 172-178.

Hendrikse, A. (1986): Intra- and interspecific sex-pheromone communication in the genus *Yponomeuta. Physiol. Entomol. 11,* 159-169.

Hendrikse, A. and E. Vos-Bünnemeyer (1987): Role of host-plant stimuli in sexual behaviour of small ermine moths (*Yponomeuta*) *Ecol. Entomol. 12,* 363-371.

Herrebout, W. M., H. J. Dijkerman and M. C. M. DeJong (1987): Stippelmot op lijsterbes: voor ons land een nieuwe combinatie? *Ned. Bosbouwtijdschr. 59,* 211-214 (In Dutch with an English summary)

Herrebout, W. M., F. J. S. W. Körner and R. W. van der Linden (1984): Time sampling traps designed for small ermine moths (Lepidoptera: Yponomeutidae) *Med. Fac. Landbouww. Rijksuniv. Gent 49,* 727-735.

Herrebout, W. M., S. B. J. Menken, G. D. E. Povel and T. P. M. van de Water. (1982): The position of *Yponomeuta yanagawanus* Matsumura (Lepidoptera, Yponomeutidae). *Neth. J. Zool. 32,* 313-323.

Herrebout, W. M. and T. P. M. van de Water (1982): The effect of the hostplant on pheromone communication in a small ermine moth *Yponomeuta cagnagellus. Med. Fac. Landbouww. Rijksuniv. Gent 47,* 503-509.

Herrebout, W. M. and T. P. M. van de Water. (1983): Trapping success in relation to height of the traps in small ermine moths *(Yponomeuta). Med. Fac. Landbouww. Gent 48,* 173-182.

Kooi, R. E. (1990): *Yponomeuta*-species and their host plants. *This volume.*

Kooi, R. E., T. P. M. van de Water and W. M. Herrebout (1989): Host selection by the so-called host races of *Yponomeuta padellus.* (in prep.)

Löfstedt, C., B. S. Hanson, H. J. Dijkerman and W. M. Herrebout (1989a): Behavioural and electrophysiological activity of unsaturated analogues of the pheromone tetradecyl acetate in the small ermine moth *Yponomeuta rorellus. Physiol. Entomol.* (in press)

Löfstedt, C., W. M. Herrebout and J. W. Du (1986): Evolution of the ermine moth pheromone tetradecyl acetate. *Nature 323,* 621-623.

Löfstedt, C., W. M. Herrebout and S. B. J. Menken (1989b): Sex pheromones and their potential role in the evolution of reproductive isolation in small ermine moths. (In prep.)

Menken, S. B. J. (1982): Biochemical genetics and systematics of small ermine moths. *Z. zool. Syst. Evol-Forsch. 20,* 131-143.

Pers, J. N. C. van der (1981): Comparison of electroantennogram response spectra to plant volatiles in seven species of *Yponomeuta* and in the tortricid *Adoxophyes orana. Entomol. exp. appl. 30,* 181-192.

Pers, J. N. C. van der (1982): Comparison of single cell responses of antennal sensilla trichodea to sex attractants in nine European small ermine moths (*Yponomeuta* spp.). *Entomol. exp. appl. 31,* 255-264.

Pers, J. N. C. van der, G. Thomas and C. J. den Otter (1980): Interaction between plant odours and pheromone reception in small ermine moths. *Chem. Senses 5,* 367-371.

Peterson, S. C., W. M. Herrebout and R. E. Kooi (1989): Chemosensory basis of host colonization by small ermine moths (*Yponomeuta*) caterpillars. (in prep.)

Povel, G. D. E. (1986): Pattern detection within the *Yponomeuta padellus*- complex. I. Biometric description and recognition of groups by numerical taxonomy. *Proc. Kon. Ned. Akad. Wet. (C) 89,* 425-441.

Povel, G. D. E. (1987a): Idem. II. Procedural conditions for the estimation of shape-differences. *Proc. Kon. Ned. Akad. Wet. (C) 90,* 367-386.

Povel, G. D. E. (1987b): Idem. III. Phenetic classification of the imagines. *Proc. Kon. Ned. Akad. Wet. (C) 90,* 387-401.

Povel, G. D. E. and W. M. Herrebout (1986): The position of *Yponomeuta gigas* Rebel. I. Morphology and biometrics. *Proc. Kon. Ned. Akad. Wet. (C) 89,* 101-109.

Water, T. P. M. van de (1983): A hostrace of the small ermine moth *Yponomeuta padellus* in northern Europe. *Neth. J. Zool. 33,* 276-282.

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EVOLUTIONARY INTERPRETATIONS OF INSECT-PLANT RELATIONSHIPS – A CLOSER LOOK

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Les théories passent.
La grenouille rest.
Jean Rostand

ABSTRACT

Specialized feeding habits of phytophagous insects have evoked several evolutionary interpretations. Most of them ignore that selection can only "tinker" from available or newly emerging genotypes. Since host selection is basically a behavioural phenomenon, it is proposed that recent relations between insects and their host plants are essentially the outcome of the evolution of plant recognition mechanism in insects. The haphazard happenings in the evolution of insect genoms is the ultimate cause of the haphazard pattern which characterizes insect-plant relationships.

Key words: evolution, coevolution, sequential evolution, selection, competition, predation, phytophagous insects, host plants, plant defense, recognition, phylogeny, behaviour, molecular genetics, molecular evolution.

INTRODUCTION

The presumed evolutionary processes leading to recent insect-plant relationships have been the subject of many theoretical considerations during the last decades. Nevertheless, I take the liberty of discussing this issue here again and in doing so I have to recapitulate a series of assumptions and facts which are generally known. Reading even some most recent publications in this domain, one gets the impression that many authors are inclined to disregard contradictory opinions and facts or do not take the trouble to discuss them.

Each evolutionary consideration dealing with insect-plant relationships essentially tries to answer the questions: What evolutionary processes have led to the host plant specialization of phytophagous insects? Why and how did the insects specialize on those plant taxa on which they now live?

Attempts have been made by various authors to find the final answer in one or several of the following assumptions:

(1) *Coevolution.* Ehrlich and Raven (1964) and several further authors proposed a reciprocal evolutionary interaction between plants and herbivorous insects resulting in food specialization of the insects as well as in speciation and radiation in both groups of organisms. The processes involved were supposed to be *selection for plant*

defense by insect attacks and, as a complementary process: *selection of the insects by the plants* for detoxifying toxic plant chemicals and for overcoming the action of behaviourally active plant chemicals. Furthermore, *competition among phytophagous insects* forced some insect lineages to occupy new plant taxa where they were free from competitors and thus could freely radiate. Parallel phylogenetic lines between insects and plants were regarded as the result of coevolution.

(2) *Diffuse coevolution*. Coevolution of insects and plants was not a simple pairwise interaction between insect and plant species, but a multitude of species must have been involved (Fox, 1988). This can be regarded as a special case of *community evolution* (Price, 1983).

(3) Apart from any direct evolutionary interaction between plants and insects *predation* forced phytophagous insect species to specialize on plants where predation was least (Bernays, 1988; Bernays and Graham, 1988), i.e. the insects were selected for a restricted host plant range which represented an *enemy free space* (Gilbert and Singer, 1975; Lawton, 1978).

(4) The presence of the *plants selects insects* for host specialization. For example, according to Futuyma (1983b, p.241) "specialization is posited to evolve first at a behavioral level, perhaps in response to the local abundance of a particular plant species".

(5) Plant evolution provided the diverse chemical and structural background for the radiation of phytophagous insects which followed plant evolution, i.e. *sequential evolution* took place (Jermy, 1976, 1984). In its course specialists emerged mostly from specialists (see Feeny, 1975).

WHAT DO FACTS INDICATE?

It is a commonplace that evidence for evolutionary considerations is mostly circumstantial. Analyzing the phylogenetic relations of the host plants of closely related insect species is the most promising way of finding out how host plant specialization may have evolved. Two main types can be distinguished: (1) parallel phylogenetic lines, i.e. closely related insect species live on closely related plant species (genera), and (2) divergent phylogenetic relations, i.e. the host plants of closely related insect species belong to different higher plant taxa.

Parallel phylogenetic lines. Since the publication of Ehrlich and Raven's (1964) paper a number of authors strongly questioned that parallel phylogenetic lines could be regarded as indirect evidence of coevolution (e.g. Jermy, 1976,1984; Janzen, 1980; Futuyma, 1983a; Mitter and Brooks, 1983). Nevertheless, even in most recent papers dealing with such types of relations "the ghost of coevolution past" is invoked. For example, the most extensively studied *Passiflora*-Heliconiine complex which is regarded by many authors as a copybook example of coevolution, has been thoroughly discussed recently by Spencer (1988) who claimed that (p.174): "The degree of correlation between heliconiine taxa ... and *Passiflora* taxa ... is indicative of a coevolutionary process, that is, a reciprocal coadaptation ... of pairs of taxa".

Space does not allow a detailed discussion of Spencer's paper, however, even a few points may clearly indicate the vagueness of such interpretations. Firstly, from the data given in the paper it is clear that the number of possible host *Passiflora* species per Heliconiine spp. varies enormously. For example, *Heliconius erato*, *Agraulis vanillae*, and *Dryas julia* may feed on 37, 35, and 26 *Passiflora* spp., respectively, while only 8 Heliconiine spp. out of 51 are monophagous. On the other hand, *Passiflora coccinea* is the potential host of 15 Heliconiine spp. that belong to 5 genera! Furthermore, two related Heliconiine spp. may feed on *Passiflora* spp. that belong to unrelated subgenera. Thus, with so many exceptions one can hardly claim a correlation of taxa.

Secondly, Spencer's main finding, namely, that oligophagous or monophagous Heliconiine spp. are able to handle only special groups of secondary plant chemicals simply means that these insects became highly specialized and adapted to the special compounds, but it does not prove that the *production* of the special plant chemicals and the evolution of the insect's *tolerance* to them resulted from a pairwise coadaptation or even from a gene-for-gene coevolutionary process as it is supposed by Spencer. The above criticism applies to all coevolutionary interpretations that are based on parallel phylogenetic lines and/or consider chemical similarities of the host plants of related insect species as proofs of coevolution. All such kind of relations may have come into being simply by sequential evolution (Jermy, 1976, 1984), i.e. colonization (Mitter and Brooks, 1983).

Divergent phylogenetic relations. In a vast number of cases closely related insect species live oligo- or monophagously on plant species that belong to different plant families or even orders (see Jermy, 1984 for references) as shown in the following examples.

From the species of the lepidopteran genus *Yponomeuta*, the species *euonymellus* lives on *Prunus padus* (Rosaceae), *rorellus* on *Salix* spp. (Salicaceae), *irrorellus* and *plumbellus* on *Euonymus* spp. (Celastraceae) and *20-punctata* on *Sedum* spp. (Crassulaceae) (Menken, 1982; and further references in Herrebout, this volume). The plant families involved belong to 3 different plant orders. It is also noteworthy that there is no correlation between the evolutionary time, i.e. the genetic distance, that separates two *Yponomeuta* species and the phyletic relations of their host plants. For example, *euonymellus* and *rorellus* between which the genetic distance is relatively short, live on very different plant taxa, while *irrorellus* and *plumbellus* between which the genetic distance is about two and a half times greater than between the former two species, live on the same host plant: *Euonymus* (Menken, 1982).

Among the aphids *Aulacorthum solani s.str.* is polyphagous, while the morphologically almost identical subspecies *A. solani langei* lives monophagously on *Pulmonaria officinalis* (Boraginaceae) and *A. solani aegopodi* on *Aegopodium podagraria* (Umbelliferae) (Müller, 1985).

From the scale insect genus *Chionaspis* the species *austriaca* occurs oligophagously on *Abies* spp. and *Pinus* spp. (Pinaceae), *Ch. lepinei* on *Quercus* spp. and *Castanea sativa* (Fagaceae), but the very closely related *Ch. salicis* is highly polyphagous oc-

curring on deciduous tree and shrub species from 17 plant families that belong to several plant orders (Kosztarab and Kozár, 1988).

In Central Europe the following species of the fruitfly genus *Rhagoletis* occur: *alternata* on *Rosa* spp. (Rosaceae), *Meigeni* and *berberidis* on *Berberis* spp. (Berberidaceae), and *cerasi* on *Prunus* spp. (Rosaceae) and on *Lonicera* spp. (Caprifoliaceae) (Mihályi, 1960). The populations of *cerasi* living on the two host plants are probably isolated genetically (Boller et al., 1976).

The Chrysomelid genus *Crioceris* comprises in Central Europe two subgenera and 6 species (Kaszab, 1962). All four species of the subgenus *Crioceris s.str.* (*asparagi*, *5-punctata*, *12-punctata*, and *14-punctata*) are monophagous on *Asparagus officinalis*. Often adults and larvae of several species can be found feeding on the same individual plant. The two species of the subgenus *Lilioceris* (*lilii*, *merdigera*) are oligophagous on various species of *Liliaceae*, but do not feed on *Asparagus*.

Similar examples of divergent phylogenetic relations can be found in a number of families of Homoptera, Coleoptera, Lepidoptera, Diptera, and Hymenoptera (see Jermy, 1984).

What do the divergent phylogenetic relations indicate?

(1) In most cases it would be more than a naivety trying to find common chemical or other features of the host plants belonging to different higher plant taxa (Mitter and Brooks, 1983).

(2) Such relations unequivocally negate *coevolution* sensu Ehrlich and Raven (1964), because the evolution of the insect species and plant species involved cannot have resulted from reciprocal selection.

(3) Following the logic of Ehrlich and Raven's (1964) concept of coevolution, Futuyma and Moreno (1988) supposed that selective agents, among others *competition*, may have resulted in the pattern of host relations shown in *Yponomeuta* species. Such an invocation of the "ghost of competition past" (Connel, 1980) may sound very attractive, but it does not explain anything, especially because interspecific competition among phytophagous insects is lacking or very rare (see Jermy, 1985, for references). The example of the four *Crioceris* species living together on *Asparagus* clearly indicate that during probably millions of years of speciation these species did not change their host plant specificity although they must have been exposed to severe interspecific competition on their only host. The same applies to *Yponomeuta irrorellus* and *Y. plumbellus* etc. having a common host.

(4) Fox (1988, p. 906) emphasized that "the concept of *diffuse coevolution* has been more successful in drawing attention to the complexity of biotic components in evolutionary interactions than in producing clear predictions of outcomes". Clearly, this concept does not provide any foot-hold for explaining why and how a certain insect species became specialized exactly to that plant species on which it now lives. Proofs for such hypotheses are the more difficult to be found since -as Janzen pointed out (1980) - in most cases it cannot be clarified, whether recent insect species and their host plants evolved together in space and time or met only after they had reached more or less their recent traits? For example, many species forming most communities in Europe may be much older than the plant communities they inhabit,

because the latter were formed mostly after the last glaciation. From extensive studies on a three-trophic-level system on willow, Craig et al. (1988, p.74) concluded that "plant herbivore interactions are typically complex and contain many forces preventing coevolution."

(5) It is most unlikely that, for example, the *Yponomeuta* species which have a similar bionomy and, therefore, must have had many common natural enemies also in the past, were forced by *predation* past to specialize on each of their present host plants, most of which now occur together in the same community. Or, the area of distribution of the four *Crioceris* species living monophagously on *Asparagus* overlap only partly, therefore, they must have been exposed to very different selection agents, among others to various forms and intensity of predation. It would be unfounded to suppose that predation made them specialize on *Asparagus* or that it maintained this specialization in a region which spreads from Western Europe to East Siberia. Or, how could predation force *Aulacorthum solani langei* to specialize on *Pulmonaria officinalis*, and *A. solani aegopodi* on *Aegopodium podagraria*, i.e. to plants which often occur in the same plant community, while *A. solani s.str.* remained polyphagous?

Bernays (1988, and this volume) has convincingly demonstrated that under experimental conditions specialized phytophagous insects are less prone to predation by generalist predators than polyphagous ones, but this proves by no means that host plant specialization was caused by predation past.

(6) The assumption of the *selection pressure exerted by the plants* on the insects resulting in host specialization can hardly explain why, for example, *Chionaspis austriaca* and *Ch. lepinei* became or remained specialists, while *Ch. salicis* occurs on plant species of 17 families; etc.

That plants with more diverse architectures select for more specialists than less diverse ones seems to hold only when total insect faunas of plants with different architectures are considered (Strong et al. 1984). However, the picture is different if plant species with similar architectures are compared . For example, on the two common oak species which form mixed populations in Hungarian forests, the numbers of cynipid species (Hymenoptera, Cynipidae) causing bud-galls are as follows: 44 species belonging to 5 genera on *Quercus robur* and 9 species from 6 genera on *Quercus cerris* (Ambrus, 1974). It is also noteworthy that only 2 cynipid species occur on both oak species! Thus, closely related and sympatric plant species with identical architecture have recruited very different numbers of specialized insect species indicating that plant structure itself cannot be a selective agent channelling insect specialization.

The case of the cynipid bud-galls also signifies that concepts like "niche", "niche segregation", "niche saturation" as factors of selection should be used most carefully in explaining the evolution of insect-plant relations. A closer comparison of plant species and their phytophagous insect guilds shows that there are many "vacant niches" (Price, 1983; Strong et al., 1984). Thus, Grant's (1989, p. 606) following reasoning about speciational trends does not hold in the case of phytophagous insects: "The availability or nonavailability of niches brings interspecific competition

and interspecific selection (or species selection) into the picture.... We can conclude, then, that the orientation in a speciational trend is the result of interspecific competition and selection acting on opportunistic speciation in an environmental gradient."

The species-area relationship (Strong et al., 1984) may implicitly suggest that the mere presence of a new plant species serves as a selective agent on the insects to specialize to it. However, the asymptotic nature of the recruitment curves found with introduced plants (Lawton and Strong, 1981), clearly proves that plants do not select for new insect lineages, but simply recruit those insect species from the local fauna that were preadapted to them.

(7) From the evolutionary interpretations only the hypothesis of *sequential evolution* (Jermy, 1976, 1984; Shield and Reveal, 1988) suitably explains the coming into being of all forms of recent insect-plant relationships which show such a haphazard, chaotic picture.

But what processes resulted in sequential evolution?

MOLECULAR EVOLUTION OF HOST SELECTION BEHAVIOUR

Concerning evolutionary hypotheses Slatkin (1983, p.30) pointed out that "most workers are faced with understanding the evolution of characters whose genetic basis is completely unknown." As regards the above discussed evolutionary interpretations, one can add that, except sequential evolution, all of them implicitly assume - in a neo-Darwinian way - that there is substantial genetic variation in the characters determining host specialization, therefore the latter can quickly evolve if selection is strong enough. However, as Endler and McLellan (1988) have shown in their recent review, the likelihood of mutations of different traits in any taxa is largely unknown. As regards phytophagous insects, there is no evidence whatsoever for an overall genetic variation of traits involved in host specialization (Mitter and Futuyma, 1983), so the emergence of new specific host relations cannot depend only on the direction of selection. In this connection Jacob's (1981) message should be thoroughly considered. It says that selection is not an engineer who is able to create anything, but it is rather a tinker, who may bungle new things only from available junk (see also Lewontin, 1970).

What does this mean to the evolutionary interpretation of insect-plant relationships?

It has been stated repeatedly that host selection, i.e. host plant specificity of phytophagous insects is primarily a behavioural phenomenon based on plant recognition by the insects (Dethier, 1954, 1970, 1976; Schoonhoven et al., 1977). Plants are mainly recognized by their chemistry, i.e. the information provided by the chemoreceptors and the central interpretation of this message are the proximate causes of host selection behaviour (Dethier, 1987). Naturally, also habitat recognition, i.e. habitat preference, recognition of plant structure and other recognition processes may play a role in the formation of specific insect behaviour. Furthermore, learning

processes may within inherited limits alter behaviours (Dethier, 1987; see Papaj and Prokopy, 1989, and Szentesi and Jermy, 1989, for references). Therefore in the following the term "plant recognition" is used in its broadest sense.

Since plant recognition is the *proximate* cause of host plant specificity, the molecular evolution of the insects' genomes determining host selection behaviour must logically be regarded as the *ultimate* driving force of the evolution of insect-plant relationships (Jermy, 1988; Jermy et al., 1989). It has been demonstrated that rather minor genetic changes may profoundly affect behaviour independently of whether morphological changes are also involved (Ehrman and Parsons, 1981; Bush, 1987) Molecular geneticists emphasized that a mutation involving a regulatory gene (Ayala, 1976) could affect many genes, for example those determining special patterns of behaviour, therefore, mutations changing the insects' plant recognition mechanisms could lead to new host plant relations (Bush, 1987; Dethier, 1987).

The fate of a newly emerged insect mutant characterized by new host plant specificity depends then on a series of conditions representing selection. First of all on the presence of suitable plant taxa to which the new mutant is tuned behaviourally. Naturally, a widely distributed, apparent and structurally diverse plant species would more likely be found and occupied by the new mutant which recognizes it as a host than a plant lacking such characteristics. This explains species-area and structural diversity relations.

The survival of the mutant would depend also on the genetic condition of its physiological adaptation (or preadaptedness) to the new host as a food, on the limited activity of predators (Price et al., 1980), on its relation to other members of the biological community to which the new host belongs, etc. (Jermy, 1988; Jermy et al., 1989)

In this respect the niche concept is meaningful since survival and further existence of the new insect mutant depends on the availability of a niche which provides the indispensable physiological and ecological conditions of survival. Availability, however, does not mean that a niche is *vacant*, as proposed by Grant (1989), it means rather the *quality* of the niche, because competition is largely lacking in phytophagous insects.

Thus, selection *maintains* insect-plant relations, but *does not create* new ones if the genetic basis of them is not given. As Lubischew (1969) rightly emphasized: selection is a great destroyer, but a very feeble creator. Therefore, there is no reason to ask: what ecological, physiological, etc. factors selected, for example, the *Yponomeuta* species to *become* specialized on such and such plant species? One may only ask: what selective agents *enable* and *maintain* that specialization? The basic question, namely, what is the *origin* of species specific host relations must be answered primarily by insect behaviour geneticists. Only geneticists will be able to find out in the future, e.g. why two sibling insect species are specialized to very different plant species or why a certain insect species is a narrow specialist while its close relative is polyphagous, etc.?

Following this reasoning it is clear that the haphazard pattern of phyletic relations between insects and their host plants is primarily due to the haphazard changes

occurring in the course of the molecular evolution of insect genomes. Three main types of genetic changes (mutations *sensu lato*) affecting host plant recognition can roughly be distinguished:

(1) The first ("conservative") type is characterized by *lack of changes* in host recognition while many other characters do change during speciation. For example, the *Crioceris* species living monophagously on *Asparagus* retained host plant specificity in considerable space and time scales.

(2) In the case of the second ("sliding") type the new insect lineage would recognize plant taxa as hosts the chemistry of which only *slightly* differs from the parental host and thus most likely would be related to the latter. In this way more or less parallel phyletic lines would emerge, like the *Passiflora*-Heliconiine complex.

(3) The third ("jumping") type results in insect lineages that would recognize plant taxa as hosts characterized by chemistry which *basically* differs from that of the parental host. Such mutations would lead to host races, sibling species or related species that feed on far related plant taxa. As illustrated by the *Yponomeuta* spp.

The two latter types of genetic changes may themselves serve as isolation mechanisms and thus may result in speciation, as has been shown by evolutionary behaviour genetic studies (Bush, 1987).

Naturally, all kind of intermediate types of changes may occur even within related groups of insect taxa. As for the degree of phagism, the direction of changes may be from stenophagy to euryphagy and *vice versa* as well as from stenophagy to new stenophagy. All these genetic changes result in the chaos which we experience surveying recent phylogenetic relations between insects and their host plants.

Following Jacob's (1981) reasoning one can conclude that the molecular evolution of insect genomes provides the "junk" which is either used (maintained) by the "tinker" called "selection" or must perish.

CONCLUSION

The molecular evolution of the insects' plant recognition mechanism, and the physiological and ecological adaptation to the host plants, is the ultimate process leading to various degrees of host plant specialization. The role of selection is only to either extinguish the maladapted mutants or to let establish the adapted ones. In order to understand the course of the evolution of insect-plant relationships, it is imperative to reveal the molecular evolutionary processes that occur in the insect genoms.

REFERENCES

Ambrus, B. (1974): *Cynipida gubacsok. - Cecidia Cynipidarum*. Fauna Hungariae 116. Akadémiai Kiadó, Budapest.

Ayala, F. J. (ed), (1976): *Molecular Evolution*. Sinauer, Sunderland, Massachusetts.

Bernays, E. A. (1988): Host specificity in phytophagous insects: selection pressure from generalist predators. *Entomol. exp. appl., 49*, 131-140.

Bernays, E. A. and M. Graham (1988): On the evolution of host selection in phytophagous arthropods. *Ecology, 69*, 886-892.

Boller, E. F., K. Russ, V. Vallo and G. L. Bush (1976): Incompatible races of European cherry fruit fly, *Rhagoletis cerasi* (Diptera: Tephritidae), their origin and potential use in biological control. *Entomol.exp.appl., 20*, 237-247.

Bush, G. L. (1987): Evolutionary behaviour genetics. In: M. D. Huettel, (ed), *Evolutionary Genetics of Invertebrate Behavior*. Plenum Press, New York. 1-6.

Connell, J. H. (1980): Diversity and coevolution of competitors, or the ghost of competition past. *Oikos, 35*, 131-138.

Craig, T. P., P. W. Price, K. M. Clancy, G. L. Waring and C. F. Sacchi (1988): Forces preventing coevolution in the three-trophic-level system: willow, a gall-forming herbivore, and parasitoid. In: K. C. Spencer (ed), *Chemical Mediation of Coevolution*. Academic Press, New York. 57-80.

Dethier, V. G. (1954): Evolution of feeding preference in phytophagous insects. *Evolution, 8*, 33-54.

Dethier, V. G. (1970): Chemical interactions between plants and insects. In: E. Sondheimer, and J. B. Simeone (eds), *Chemical Ecology*. Academic Press, New York. 83-102.

Dethier, V. G. (1976): The importance of stimulus patterns for host-plant recognition and acceptance. *Symp. Biol. Hung., 16*, 67-70.

Dethier, V. G. (1987): Analyzing proximate causes of behavior. In: M. D. Huettel, (ed), *Evolutionary Genetics of Invertebrate Behavior*. Plenum Press, New York. 319-328.

Ehrlich, P. R. and P. H. Raven (1964): Butterflies and plants: a study in coevolution. *Evolution, 18*, 586-608.

Ehrman, L. and P. A. Parsons (1981): *Behavior Genetics and Evolution*. McGraw-Hill, New York.

Endler, J. A. and T. McLellan (1988): The process of evolution: toward a newer synthesis. *Ann. Rev. Ecol. Syst., 19*, 395-421.

Feeny, P. P. (1975): Biochemical coevolution between plants and their insect herbivores. In: L. E. Gilbert, and P. H. Raven (eds), *Coevolution of Animals and Plants*. University of Texas Press, Austin. 3-19.

Fox, L. R. (1988): Diffuse coevolution within complex communities. *Ecology, 69*, 906-907.

Futuyma, D. J. (1983a): Evolutionary interactions among herbivorous insects and plants. In: D. J. Futuyma, and M. Slatkin. (eds), *Coevolution*. Sinauer, Sunderland, Massachusetts. 207-231.

Futuyma, D. J.(1983b): Selective factors in the evolution of host choice by phytophagous insects. In: S. Ahmad, (ed), *Herbivorous Insects: Host Seeking Behavior and Mechanisms*. Academic Press, New York. 227-244.

Futuyma, D. J. and G. Moreno (1988): The evolution of ecological specialization. *Ann. Rev. Ecol. Syst., 19*, 207-233.

Gilbert, L. E. and M. C. Singer (1975): Butterfly ecology. *Ann. Rev. Ecol. Syst., 6*, 365-397.

Gilbert, L. E. and J. T. Smiley (1978): Determinants of local diversity in phytophagous insects: host specialists in tropical environments. In: L. A. Mound, and N. Waloff (eds), *Diversity of Insect Faunas*. Blackwell Scientific, London. 89-104.

Grant, V. (1989): The theory of speciational trends. *Am. Nat., 133*, 604-612.

Jacob, F. (1981): *Le jeu des possibles*. Fayard, Paris.

Janzen, D. H. (1980): When is it coevolution? *Evolution, 34*, 611-612.

Jermy, T. (1976): Insect-host-plant relationships – co-evolution or sequential evolution? *Symp. Biol. Hung., 16*, 109-113.

Jermy, T. (1984): Evolution of insect/host plant relationships. *Am. Nat., 124*, 609-630.

Jermy, T. (1985): Is there competition between phytophagous insects? *Z. Zool. Syst. Evol.-forschung., 23*, 275-285.

Jermy, T. (1988): Can predation lead to narrow food specialization in phytophagous insects? *Ecology, 69*, 902-904.

Jermy, T., E. Lábos and I. Molnár (1989): Stenophagy of phytophagous insects - a result of constraints on the evolution of the nervous system. In: J. Maynard Smith, and G. Vida (eds), *Organizational Constraints on the Dynamics of Evolution*. Manchester University Press, Manchester. 157-166.

Kaszab, Z. (1962): *Levélbogarak. - Chrysomelidae*. Fauna Hungariae 63. Akadémiai Kiadó. Budapest.

Kosztarab, M. and F. Kozár (1988): *Scale Insects of Central Europe*. Akadémiai Kiadó, Budapest.

Lawton, J. H. (1978): Host plant influences on insect diversity: the effects of space and time. In: L. A. Mound, and N. Waloff (eds), *Diversity of Insect Faunas*. Blackwell Scientific, London. 105-125.

Lawton, J. H. and D. R. Strong, Jr. (1981): Community patterns and competition in herbivorous insects. *Am. Nat., 118*, 317-338.

Lewontin, R. C. (1970): The units of selection. *Ann. Rev. Ecol. Syst.*, 1, 1-18.

Lubischew, A. A. (1969): Philosophical aspects of taxonomy. *Ann. Rev. Entomol., 14*, 19-38.

Menken, S.B.J. (1982): Biochemical genetics and systematics of small ermine moths (Lepidoptera: Yponomeutidae). *Z. Zool. Syst. Evol. forschung, 20,* 131-143.

Mihályi, F. (1960): *Fúrólegyek. - Trypetidae*. Fauna Hungariae 56. Akadémiai Kiadó, Budapest.

Mitter, C. and D. R. Brooks (1983): Phylogenetic aspects of coevolution. In: D. J. Futuyma, and M. Slatkin (eds) *Coevolution*. Sinauer, Sunderland, Massachusetts, 65-98.

Mitter, C. and D. J. Futuyma (1983): An evolutionary genetic view of host plant utilization by insects. In: R. F. Denno, and M. S. McClure (eds), *Herbivores and their Variable Hosts in Natural and Managed Systems*. Academic Press, New York. 427-459.

Müller, F. P. (1985): Genetic and evolutionary aspects of host choice in phytophagous insects, especially aphids. *Biol. Zbl., 104*,225-237.

Papaj, D. R. and R. J. Prokopy (1989): Ecological and evolutionary aspects of learning in phytophagous insects. *Ann.Rev.Entomol. 34*, 315-350.

Price, P. W. (1983): Hypotheses on organization and evolution in herbivorous insect communities. In: Denno, R. F. and M. S. McClure (eds), *Variable Plants and Herbivores in Natural and Managed Systems*. Academic Press, New York. 559-596.

Price, P. W., C. E. Bouton, P. Gross, B. A. McPherson, J. N. Thompson and A. E. Weis (1980): Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Ann. Rev. Ecol. Syst., 11*, 41-65.

Schoonhoven, L. M., N. M. Tramper and W. van Drongelen (1977): Functional diversity in gustatory receptors in some closely related *Yponomeuta* species (Lep.). *Neth. J. Zool. 27*, 287-291.

Shields, O. and J. L. Reveal (1988): Sequential evolution of *Euphilotes* (Lycaenidae: Scolitantidini) on their plant host *Eriogonum* (Polygonaceae: Eriogonoidae). *Biol. J. Linn. Soc. 33*, 51-93.

Slatkin, M. (1983): Genetic background. In: D. J. Futuyma, and M. Slatkin (eds), *Coevolution*. Sinauer, Sunderland, Massachusetts. 14-32.

Spencer, K. C. (1988): Chemical mediation of coevolution in the *Passiflora - Heliconius* interaction. In: K. C. Spencer, (ed), *Chemical Mediation of Coevolution*. Academic Press, New York. 167-240.

Strong, D. R., J. H. Lawton and T. R. E. Southwood (1984): *Insects on Plants*. Blackwell Scientific, Oxford.

Szentesi, Á. and T. Jermy (1989): The role of experience in host plant choice by phytophagous insects. In: E. A. Bernays, (ed), *Insect-Plant Interactions*. Vol II., CRC Press, Boca Raton (in press).

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EVOLUTION OF HOST-PLANT SPECIFICITY

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ABSTRACT

Narrow host range is generally considered to have benefits nutritionally. Here, an alternative or supplementary view is taken, that generalist natural enemies provide a selection pressure for narrow host range. Data will be presented from three different systems: predatory wasps and caterpillars, ants and small caterpillars, coccinellids and aphids. These suggest that generalist herbivores are more vulnerable to predation than specialist herbivores for a variety of different reasons. From this we suggest that the predators maintain narrow host range in phytophagous insects, and that they may also be responsible in large part for the evolution of the narrow host ranges so commonly found.

Key words: specificity, host-plant range, oligophagy, predators, tritrophic interactions, evolution, deterrent, selection pressure.

Few would disagree with the following aspects of plant-insect interactions:

1. Plant chemistry plays the decisive role in the behavioral selection of a host plant by an ovipositing lepidopteran or a feeding grasshopper.

2. Volatiles, surface wax components, internal nutrients and plant secondary compounds, are all demonstrably important to differing degrees, and rejected plants generally provide chemical information that signifies their non-host status for the insects encountering them.

3. The more specific the insects, the greater the range of compounds that deter oviposition or feeding, whether or not host-specific chemicals play an important additional role of a positive nature.

4. The majority of phytophagous insect species have a very restricted host range and are thus deterred by a wide variety of plant secondary compounds.

A variety of different evolutionary pressures could have led to the patterns of host-specificity we see today and with respect to these ultimate, evolutionary pressures there is considerably less agreement. Because of the conspicuous importance of plant chemistry in behavioral selection of plants, and the fact that many plant compounds are detrimental to insects not normally ingesting them, it is natural that plant chemistry generally figures as a major player, selecting for changes in host plant use and plant specificity. I suggest that other approaches have been overlooked and that other possibilities are also likely to have been important.

Some other possible evolutionary sequences are as follows:

1. Since omnivory, fungivory and plant polyphagy are likely to have preceded specialization on particular diets, it is logical to assume that a variety of generalized tolerance mechanisms was commonly present in earlier insect species, each with its variability in expression.

2. Widely differing selective pressures provided advantages to particular populations in becoming more selective. For example, increased abilities in relation to risk avoidance are conceivable, as are improvements in nutrient acquisition, increased efficiency in mate finding or improved thermal conditions.

3. As a consequence of these advantages there would be selection for efficient discrimination among hosts that vary in the relevant character, and since the plants differ so markedly in chemistry, it would be expected that chemicals should become determinants of plant choice, allowing discrimination among potential hosts that vary in the feature of importance. Non-hosts should become unacceptable and chemically deterrent, while hosts may become specifically attractive. This could explain the fact that so many deterrents are apparently harmless (e.g. Bernays and Chapman, 1987).

4. A later step among specialized insect species would be a loss of generalized post-ingestional tolerance of plant secondary chemicals that do not occur in the hosts. This seems almost certainly to have been the sequence of events in the case of the various groups of graminivorous grasshoppers whose derivation is from highly tolerant polyphages. Thus physiological toxicity of certain non-host chemicals to specialist insects would be a result and not a cause of specialization (Bernays and Chapman, 1978).

Such a sequence of events is very different from an alternative, widely held view : insects evolve many avoidance mechanisms in order to escape the consequences of ingesting detrimental chemicals. An individual insect avoids plant "x" which contains the toxic alkaloid "y". Did the species evolve an ability to reject the plant because of the noxious effects of "y"? Or, on the contrary, did it evolve a rejection mechanism because of other types of fitness loss associated with being on "x", and *subsequently* develop a physiological sensitivity which was not originally present?

Various different approaches are necessary to estimate the relative importance of these views. Here, are presented data from a somewhat neglected area, that may bear on host plant selectivity in phytophagous insects, namely, predation (Bernays and Graham, 1988).

Predation is a common major cause of mortality, and has probably been so for most of the evolutionary history of insects. If association with particular plants confers protection on phytophages against predation, then there will be selection pressures provided by predators, influencing host-plant use. Experiments were carried out to test the hypothesis that specialist herbivores are less vulnerable to predation than generalist herbivores. The following assumptions were made:

1. Predators cause significant losses in phytophagous species, and have done so through evolutionary time.

Table 1 Preference shown by three insect predator species for appropriate prey having similar size but different host ranges (partly after Bernays, 1988; Bernays and Cornelius, 1989).

Predator	Tests	Number of experiments	Preference
Myschocyttarus flavitarsus	a: brightly colored specialists vs b: generalists	10	a: $p<0.001$
	a. green/brown specialists vs b: generalists	18	a: $p<0.01$
	a. generalists vs b: generalists	8	a=b
Iridomyrmex humilis[•]	a: brightly colored specialists vs b: generalists	10, 15	a: $p<0.01$
	a: brown/green specialists vs b: generalists	19, 15	a: $p<0.05$
Hippodamia convergens	a: specialists vs b: generalists	6	a: $p<0.05$

[•] Because tests all used a common standard experiments with a) and b) are separate

2. Phytophages have sufficient genetic variability and other bases for lability that changes in host plant preference and host shifts are common.

Extensive tests were carried out with two generalist predators and a range of Lepidoptera with different larval host ranges. The predators were the vespid, *Myschocyttarus flavitarsus* and the ant, *Iridomyrmex humilis*. The former was examined in relation to populations of caterpillars approximately 1.5 cm in length, feeding on their host plants and available in species pairs of similar density but differing host range (Bernays, 1988). The ant was tested with caterpillars of less than 5 mm in length, with each test species being examined for acceptability to foraging workers in relation to a common standard (Bernays and Cornelius, 1989). Further tests were carried out with the aphid predator *Hippodamia convergens*, and a series of aphids having differing host ranges (Bernays, in prep.).

In each of the three systems, the generalist herbivores were significantly more vulnerable to predation than the specialists (Table 1). The reasons were varied, and in some cases could not be determined. Among the relative specialists were noted such things as greater degrees of repellence, deterrence, crypsis and/or effective escape behavior. Taken together, the data strongly imply that one of the significant benefits of relatively narrow host range is protection from generalist predators. It is possible that many ways of using host plant features for protection may be fine-tuned in specialists.

An examination of the literature has revealed considerable evidence for the relative vulnerability of generalist herbivores to predation, in comparison with species having narrower host ranges (e.g. Brower, 1958; Heads and Lawton, 1984, 1985; Jaenike, 1985; MacLean et al., 1989; Moran, 1986a,b; Pierce and Elgar, 1985). It is suggested here that further studies on differential fitness in relation to host range should be examined in a variety of ecological contexts, and populations of species that vary in host range.

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REFERENCES

Bernays, E. A. (1988): Host specificity in phytophagous insects: selection pressure from generalist predators. *Entomol. exp. appl. 49,* 131-140.

Bernays, E. A. and R. F. Chapman (1978): Plant chemistry and acridoid feeding behaviour. In: J. B. Harborne (ed), *Coevolution of Plants and Animals.* Academic Press, New York. 100-141.

Bernays, E. A. and R. F. Chapman (1987): Evolution of deterrent responses by phytophagous insects. In: R. F. Chapman, E. A. Bernays and J. G. Stoffolano (eds), *Perspectives in Chemoreception and Behavior.* Springer-Verlag, New York. 159-174.

Bernays, E. A. and M. Cornelius (1989): Generalist caterpillar prey are more palatable than specialists for the generalist predator *Iridomyrmex humilis. Oecologia 79,* 427-430.

Bernays, E. A. and M. Graham (1988): On the evolution of host specificity in phytophagous arthropods. *Ecology 69,* 886-892.

Brower, L. P. (1958): Bird predation and foodplant specificity in closely related procryptic insects. *Am. Nat. 92,* 183-187.

Heads, P. A. and J. H. Lawton (1984): Bracken, ants and extrafloral nectaries. II. The effect of ants on the insect herbivores of bracken. *J. Anim. Ecol. 53,* 1015-1932.

Heads, P. A. and J. H. Lawton (1985): Bracken, ants and extrafloral nectaries. III. How insect herbivores avoid ant predation. *Ecol. Entomol. 110,* 29-42.

Jaenike, J. (1985): Parasite pressure and the evolution of amanitin tolerance in *Drosophila. Evolution 39,* 1295-1301.

MacLean, D. B., T. D. Sargent and B. Maclean (1989): Discriminant analysis of Lepidopteran prey characteristics and their effects on the outcome of bird-feeding trials. *Biol. J. Linn. Soc. 36,* 295-311.

Moran, N. A. (1986a): Morphological adaptation to host plants in *Uroleucon* (Homoptera: Aphididae). *Evolution 40,* 1044-1050.

Moran, N. A. (1986b): Benefits of host plant specificity in *Uroleucon* (Homoptera: Aphididae). *Ecology 67,* 108-115.

Pierce, N. E. and M. A. Elgar (1985): The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilous lycaenid. *Behav. Ecol. Sociobiol. 16,* 209-222.

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PLANT ECOTONES AND BUTTERFLY SPECIATION: BIOLOGICAL OR PHYSICAL CAUSES

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ABSTRACT

The Great Lakes region of North America contains an ecotone between boreal and temperate deciduous forests. For many species of Lepidoptera including *Papilio glaucus*, this zone marks the boundary between closely related taxa. We have recently documented numerous differences in wing morphology, colour, diapause, food plant usage abilities, allozymes, mitochondrial DNA, and larval characters between the *glaucus* and *canadensis* subspecies. Our goal is to determine the relative importance of abiotic variables (e.g. climatic regimes) vs. biological interactions (e.g. host affiliations) in maintaining this zone of disjunction. We hope to develop and employ geographic information systems to precisely predict particular localities within the ecotone where hybridization is likely to occur. The detailed description of the *glaucus/canadensis* hybrid zone resulting from this study will provide a sensitive baseline for comparisons with other species in this zone, and for assessing the biotic effects of environmental perturbations such as climatic warming.

Key words: genetics, *Papilio glaucus*, hybrid zone, diapause, voltinism, *Liriodendron tulipifera*, *Populus tremuloides*, detoxification, allozymes, mitochondrial DNA, oviposition preference, introgression, linkage disequilibrium, sexual selection, polymorphism, mimicry.

The Great Lakes region of North America contains a well-known plant transition zone between boreal and temperate deciduous forests (Curtis, 1959). An unusual number of disjunctions in the distributions of animal taxa occurs in the same general area (Remington, 1968). For the tiger swallowtail, *Papilio glaucus*, and dozens of Lepidoptera species, this suture zone across Michigan and Wisconsin marks the transition from one form to another (Platt, 1983; Opler and Krizek, 1984; Scriber and Hainze, 1987). Our continuing goal is to determine the relative importance of abiotic variables (e.g. climatic regimes) versus biological interactions (e.g. host affiliations and mating interactions) in maintaining this zone of disjunction between *Papilio glaucus glaucus* and *P. g. canadensis*.

Although specific biochemical and physiological phenotypes often have a direct effect on "fitness" in a particular environment, their genetic basis is rarely known. While experimental manipulation of the environments (e.g. A + B, Fig. 1) is possible, it is much less likely that experimental exchanges of genes will be possible (Fig. 1). The utility of the natural hybrid zones of *P. glaucus* is therefore great.

Hybrid zones are defined as "narrow regions in which genetically distinct populations meet, mate, and produce hybrids" (Barton and Hewitt, 1985). They may reflect

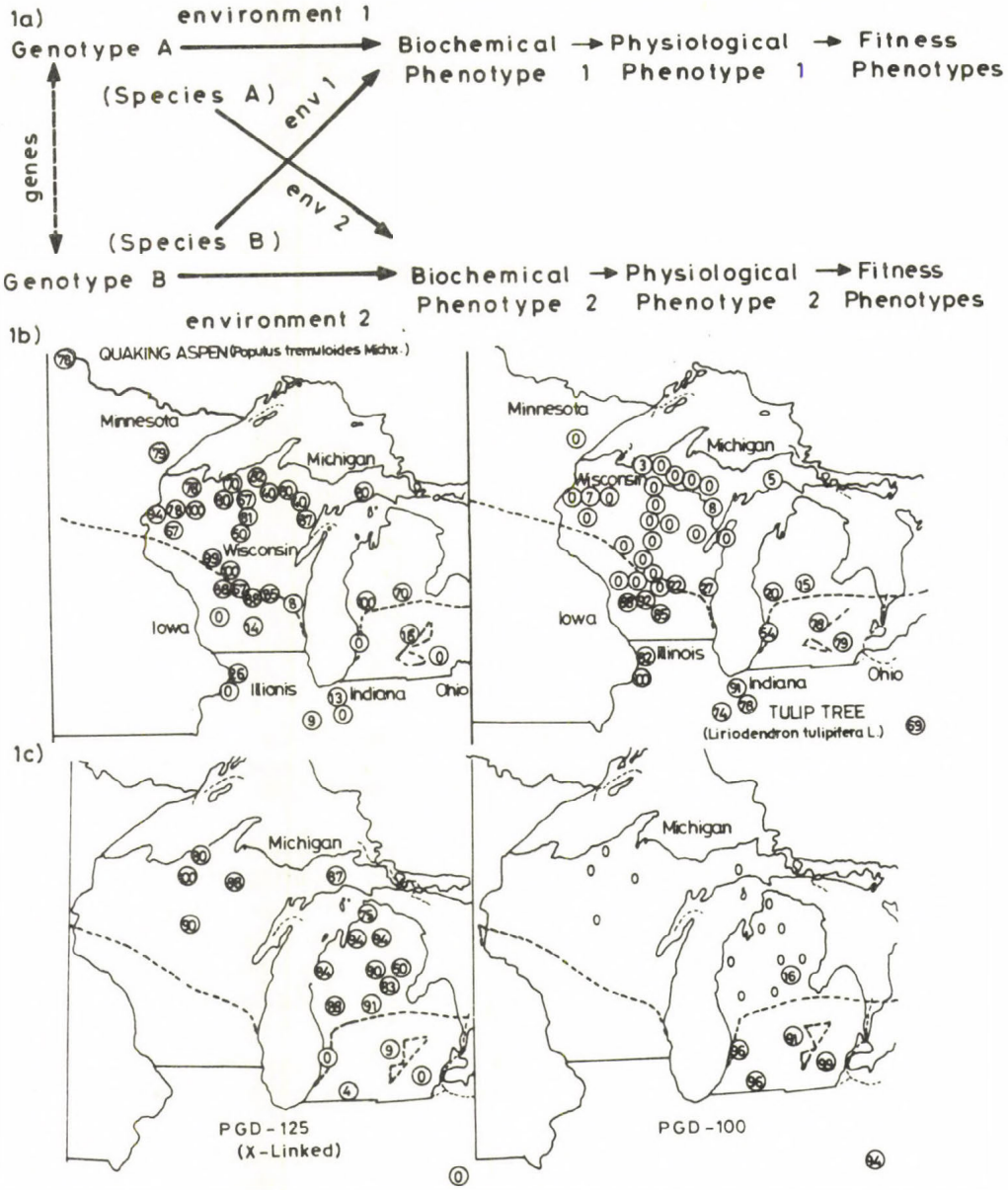

Fig. 1a Experimental procedures for determining relative contribution of different genotypes to physiological performance and to assess their contribution to the evolutionary success of individuals (after Koehn, 1987).

Fig. 1b Percent neonate survival of *P. glaucus* populations on aspen and tulip tree.

Fig. 1c Frequency of one sex-linked diagnostic allozyme for male *P. glaucus*. Dotted line is the 1444°C thermal degree day accumulation.

secondary contact of populations following allopatric isolation and leading either to coalescence or complete reproductive isolation (Mayr, 1963), or they may arise as intermediate stages in a parapatric speciation event (Diehl and Bush, 1984).

Yet evidence indicates that hybrid zones can remain remarkably stable across millennia. The prevailing explanation is that they represent a balance between dispersal into the hybrid zone and selection against hybrids within the zone (Barton and Hewitt, 1985). Inferior hybrids are maintained by the crossbreeding of naive parentals that immigrate into the zone of contact from allopatric pure populations. One alternative view is that hybrid zones are due to the co-occurrence of parallel environmental gradients (Moore, 1977). Here maintenance of a zone is not due to hybrid unfitness *per se.* In this case, clines should be both broader and not so closely positioned for all loci involved. A subset of this view is that hybrids are actually superior to either pure parental population in a particular novel or ecotonal environment (Collins, 1984). In this situation, reproductive isolation should decrease within the ecotonal area.

These alternative hypotheses lead to specific predictions that are testable within the context of *P. glaucus*. The chief contrasts involve (1) relative fitness of hybrids and parental types in different environments, (2) concordance of change in allelic frequencies and other genetically-based traits through the transition zone, and (3) the presence or absence of reproductive isolation and reinforcement.

P. glaucus is common from the boreal forests of central Alaska and Canada south and eastward into southern Florida, occupying a one billion hectare geographic range. Populations intermediate between *glaucus* and *canadensis* occur along a band between 40° and 45°N latitude, which corresponds generally with the temperate-deciduous/boreal-coniferous forest ecotone in eastern North America (Hagen, in press; Luebke et al., 1988). Hybridization between the subspecies occurs, but its frequency and geographic extent have not been quantified. Our overall research goal is to characterize the precise geographic distribution across the Michigan forest ecotone for at least a dozen genetically-based traits differing between *glaucus* and *canadensis* (Table 1). The degree concordance in distribution of these key traits will indicate the relative importance of environmental factors versus hybrid breakdown in delineating the northern limits to *P. g. glaucus* gene flow and the southern limits to *P. g. canadensis* gene flow.

METHODS

The protocol of multi-trait data collection is complex, but efficient (Fig. 2). Counting the mitochondrial DNA and three allozymes, we can assess up to 8 genotypic characteristics of each male. Field-collected females are handled using a similar protocol.

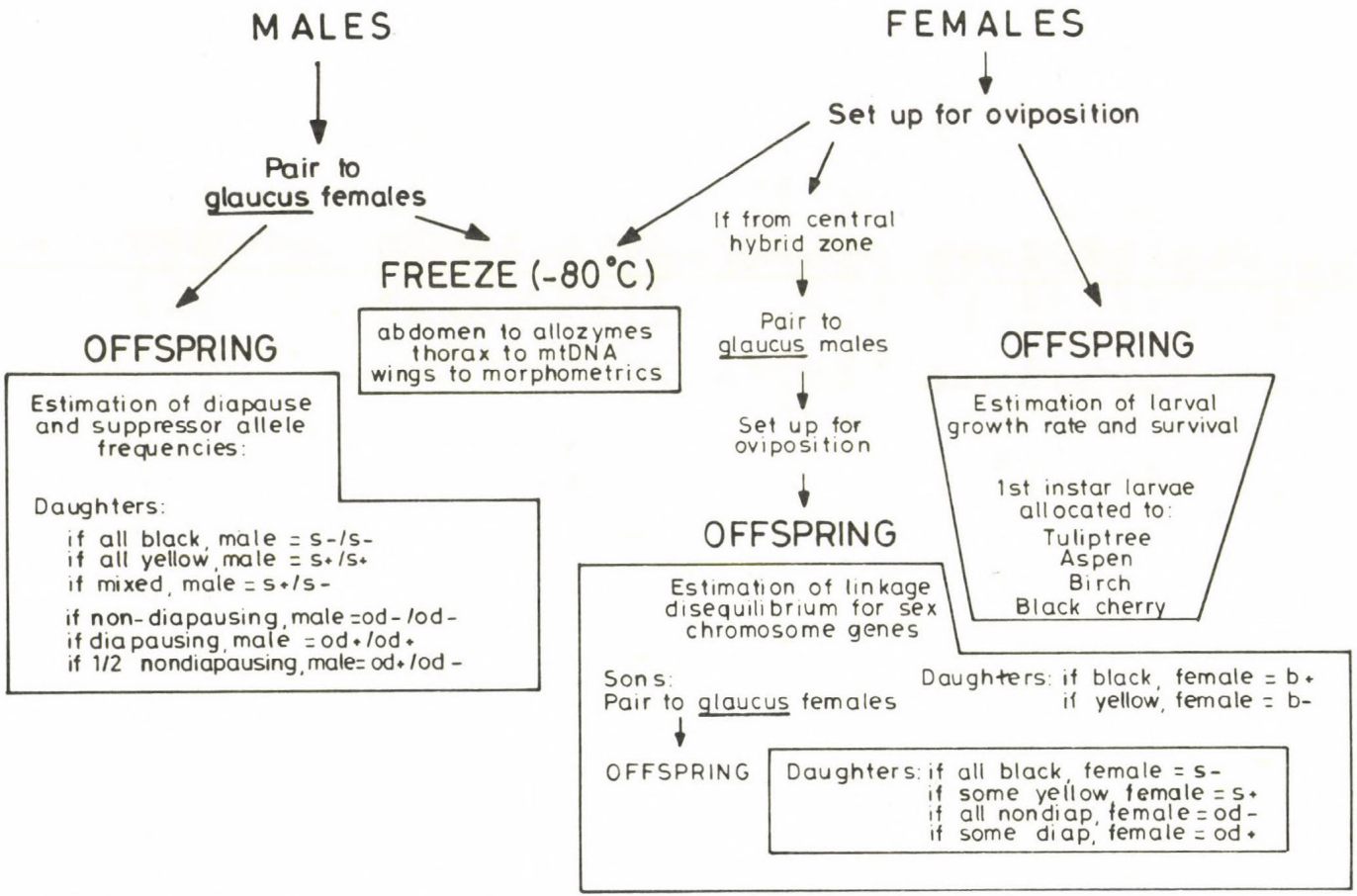

Fig. 2 Protocol for processing wild caught butterflies sampled from populations along the hybrid zone transects in Michigan and Wisconsin. (s+ and s- are *canadiensis* and *glaucus* alleles respectivelly at the X-linked black morph suppressor locus; od+ and od- are *canadiensis* and *glaucus* alleles at the X-linked diapause locus; mtDNA is mitochondrial DNA.)

Table 1 Summary of genetic differences *P. g. glaucus* and *P. g. canadensis*

	P. g. glaucus trait	*P. g. canadiensis* trait	Mode of inheritance	Reference
1.	Ldh 100	Ldh 79	x-linked allozyme	Hagen and Scriber, 1989
2.	Pgd 100	Pgd-125	x-linked allozyme	Hagen and Scriber, 1989
3.	Hk fast	Hk slow	autosomal allozyme	Hagen and Scriber, in prep.
4.	n=4 fragments (enzyme X ba I)	n=3 fragments	cytoplasmic (mt DNA)	Hagen, Smith and Scriber, in prep.
5.	Obligate diapause	Obligate diapause	single locus recessive? x-linked	Rockey et al., 1987a Hagen and Scriber, 1989
6.	female melanism		single y-linked locus	Scriber et al., 1987; Hagen and Scriber, 1989
7.		melanism suppression	single x-linked locus	Scriber et al., 1987; Hagen and Scriber, 1989
8.	Tuliptree detoxification		2-4 loci? dominant autosomal?	Scriber, 1986; Scriber et al., 1989
9.		Quaking aspen detoxification	2-4 loci? dominant autosomal?	Scriber, 1986
10.		Paper birch detoxification	?	Scriber et al., unpubl.
11.	Large adults	Small adults	Polygenic?	Luebke et al., 1988
12.	Prefers tuliptree for oviposition	Prefers aspen for oviposition	?	Scriber et al., in prep. (see also Table 2)

RESULTS

1. Larval performance. Because of overlap of southern and northern hosts across the Great Lakes ecotone, foodplants for *P. glaucus* are most numerous in the central Michigan region. Steep clines in the ability of *P. glaucus* larvae to use key host plants suggest that selection acting on the larvae may play a critical role in the distribution of *glaucus* and *canadensis* types (Scriber, 1986; Lindroth et al., 1988). In particular the Betulaceae and Salicaceae are excellent for *P. g. glaucus*, with reciprocal inabilities demonstrated for quaking aspen and tuliptree (Fig. 1b). The transition between subspecies corresponds approximately to the northernmost limit of tuliptree, *Liriodendron tulipifera,* and the southernmost limit of trembling aspen, *Populus tremuloides*, and paper birch, *Betula papyrifera*. Each host species is used frequently by tiger swallowtails where it occurs, but *P. g. glaucus* rarely encounters aspen or birch and *P. g. canadensis* rarely encounters tuliptree.

2. Diapause and voltinism. The number of generations per year (voltinism) produced by *P. glaucus* is determined by pupal diapause. Over 95% of *canadensis* indi-

Table 2 Preliminary oviposition results of *P. g. canadensis*, *P. g. glaucus* and individuals (*P. g."subspecies"*) from the Michigan ecotone. A 3-choice arena (clear plastic box with tuliptree, *Liriodendron tulipifera*; black cherry, *Prunus serotina*; and quaking aspen, *Populus tremuloides*) was illuminated in alternating 6 hr. periods. These studies were of a 3 day duration with leaf position rotations each 24 hours. These pilot studies were all done in the first 2 weeks of June, 1989 and are limited in sample size, but suggest that a very different response exists with respect to *P. g. canadensis* and *P. g. glaucus* on aspen and tuliptree.

Subspecies and location	No. of females	Percent of eggs (3 day total)			Total eggs (n)
		Tuliptree	Black cherry	Quaking aspen	
*P. g. canadensis** (n=4 counties from northern MI/WI) MEAN±S.E.	4	24.0±12.0	21.2±7.7	55.0±14.9	(302)
*P. g. (subspecies)*** (n=3 counties from south central Michigan) MEAN±S.E.	11	62.2±6.1	29.3±7.8	8.5±3.7	(744)
*P. g. glaucus**** (from IL, KY, OH, GA, FL.) MEAN±S.E.	19	78.9±3.6	12.7±2.2	8.3±2.2	(1415)

Not included in the table:
* Nine *P. g. canadensis* laid fewer than 10 eggs;
** Five Ingham Co. females laid fewer than 10 eggs;
*** One Florida female, one Georgia female, and three Ohio females each laid fewer than 10 eggs.

viduals undergo pupal diapause regardless of the rearing environment (Rockey et al., 1987a, b). In contrast, the incidence of diapause among *glaucus* in the laboratory is strongly affected by rearing environment: it is decreased by long photoperiod, warm temperatures (Rockey et al., 1987b) and, possibly, high quality larval food (Hagen and Lederhouse, 1985). Under identical laboratory conditions, the frequency of diapause is also lowest for the most southerly *glaucus* populations (Hagen and Lederhouse, 1985; Rockey et al., 1987b). This presumably reflects genetic variation in the thresholds for diapause induction within *glaucus*, which is lacking in *canadensis*.

The transition from *glaucus* to *canadensis* is located approximately by the seasonal isotherm corresponding to the minimum "degree-day" requirement for completion of two generations by *P. glaucus* (ca. 1444-1555°C; Scriber and Lederhouse, 1983; Ritland and Scriber, 1985). Populations occurring north of the isotherm (*canadensis*) are limited by climate to a univoltine life cycle (one generation per year), while populations to the south (*glaucus*) have the potential to be multivoltine (Hagen and Lederhouse, 1985; Scriber, 1988).

3. Oviposition preferences. Over most of the *P. glaucus* range, 4 to 24 suitable hosts are present, and local specialization suggests female choice of host species in a given area. In laboratory tests with individual females from north, south and throughout the Michigan ecotone, we have found a distinct difference in subspecies preference for tuliptree in the south and quaking aspen in the north (see Table 2).

4. Allozyme differences. Two loci are X-linked in the *P. glaucus* subspecies: Ldh (lactate dehydrogenase) and Pgd (6-phosphogluconate dehydrogenase) (Hagen, in press; Hagen and Scriber, 1989; Fig. 1c). That these sex-linked allozyme loci differ between *glaucus* and *canadensis* is especially intriguing. It is unlikely that the association is due solely to chance, since the haploid chromosome number for *P. glaucus* is probably 31. In addition, one autosomal allozyme locus, Hk (hexokinase), has been found to differ significantly between the subspecies (Hagen, 1989; Hagen and Scriber, in prep.).

Previous studies provide strong, though indirect, evidence for parallel clines in the direction of selection on diapause and female color phenotypes in *P. glaucus* (Hagen and Lederhouse, 1985; Ritland and Scriber, 1985). Linkage between selected loci can intensify barriers to gene flow across a hybrid zone (Barton and Hewitt, 1985). Alleles at the diapause and female color loci will be directly affected by this selection gradient — which may be sufficient to prevent free exchange of neutral alleles at other linked loci. Thus, Pgd allozymes of *canadensis* and *glaucus* need not be under selection to account for their clinal distribution. How much of the X-chromosome can be subject to the same influence is uncertain: for example, Ldh may be too far away from the diapause-Pgd-female color complex for hitchhiking to account for its allele frequency cline.

5. Sexual Selection and Color Polymorphism. Glaucus, but not *canadensis*, exhibits a female-limited polymorphism for Batesian mimicry of the unpalatable pipevine swallowtail butterfly, *Battus philenor*. Sexual selection has been suggested as a mechanism opposing spread of the black phenotype outside of the model's range (Burns, 1966), but this proposal remains controversial (Platt et al., 1984; Lederhouse et al., 1989). Research currently in progress in this laboratory is intended, in part, to address this question. Female color in *glaucus* is maternally inherited, with few exceptions (Clarke and Sheppard, 1962; Scriber et al., 1987). Hybridization of *glaucus* females with *canadensis* males, however, results in yellow daughters regardless of maternal phenotype (Scriber et al., 1987). Yellow daughters from black mothers will produce black daughters if mated to *glaucus* or hybrid males, indicating that the results are due to reversible suppression of the black phenotype. *Canadensis* populations appear to be fixed both for the X-linked suppressor and the yellow color allele on the Y chromosome (Scriber, Hagen and Lederhouse, in prep.).

Our preliminary evidence suggests that courting *glaucus* males prefer yellow females to dark females where dark females are rare. Male preference for female color or size might reduce hybridization or determine the direction of potential crosses where the generally larger *glaucus* and smaller *canadensis* are parapatric. If males prefer larger females, crosses between *glaucus* males and *canadensis* females might be rarer than expected. Male color or size preference might be a component of possible reinforcement of reproductive isolation. Similarly, females needing an additional spermatophore (Lederhouse and Scriber, 1987) might direct significantly more courtship solicitation flights toward males of their own subspecies than the other subspecies.

CONCLUSIONS

The species richness of biological communities at ecotones is a fascinating and important phenomenon for aesthetic as well as practical reasons. With a greater understanding of the dynamics of the boreal/temperate ecotone across the Great Lakes region of North-America, we will be better able to assess the importance of tight host affiliations versus physical (abiotic) factors in limiting the geographic distribution of insects. With known genetically-based characters differentiating *glaucus* and *canadensis* and the tools of allozyme electrophoresis, mitochondrial DNA analysis, and the computerized geographic information system (G.I.S.), we should have the power to accurately track variations in a suite of genetically-based characters across this transition zone through space and time. This may provide an extremely precise and temporally responsive biological monitoring device for tracking short-term climatic warming trends.

REFERENCES

Barton, N. H. and G. M. Hewitt (1985): Analysis of hybrid zones. *Ann. Rev. Ecol. Syst. 16,* 113-148.

Burns, J. M. (1966): Preferential mating versus mimicry: Disruptive selection and sex-limited dimorphism in *Papilio glaucus*. *Science 153,* 551-553.

Clarke, C. A. and P. M. Sheppard (1962): The genetics of the mimetic butterfly *Papilio glaucus*. *Ecology 43,* 159-161.

Collins, M. M. (1984): Genetics and ecology of a hybrid zone in *Hyalophora* (Lepidoptera: Saturniidae). *Univ. Calif. Publ. Entomol. 104,* 1-93.

Curtis, J. T. (1959): *The vegetation of Wisconsin*. Univ. of Wisconsin Press, Madison.

Diehl, S. R. and G. L. Bush (1984): An evolutionary and applied perspective of insect biotypes. *Ann. Rev. Entomol. 29,* 471-504.

Hagen, R. H. (1989): Population structure and host use in hybridizing subspecies of *Papilio glaucus* (Lepidoptera: Papilionidae). *Evolution* (in press)

Hagen, R. H. and R. C. Lederhouse (1985): Polymodal emergence of the tiger swallowtail, *Papilio glaucus* (Lepidoptera: Papilionidae): Source of a false second generation in central New York State. *Ecol. Entomol. 10,* 19-28.

Hagen, R. H. and J. M. Scriber (1989): Sex-linked diapause, color and allozyme loci in the tiger swallowtail butterfly: linkage analysis and implications for hybrid zone biology. *J. Heredity 80,* 179-185.

Koehn, R. K. (1987): The importance of genetics to physiological ecology. In: M. E. Feder, A. F. Bennett, W. W. Burggren and R. B. Huey (eds), *New Directions in Ecological Physiology.* Cambridge University Press, Cambridge. 170-185.

Lederhouse, R. C., M. P. Ayres and J. M. Scriber (1989): Evaluation of spermatophore counts in studying mating systems of Lepidoptera. *J. Lepidopt. Soc. 43,* 93-101.

Lederhouse, R. C. and J. M. Scriber (1987): Ecological significance of a postmating decline in egg viability in the tiger swallowtail. *J. Lepidopt. Soc. 41,* 83-93.

Lindroth, R. L., J. M. Scriber and M. T. S. Hsia (1988): Chemical ecology of the tiger swallowtail: mediation of host use by phenolic glucosides. *Ecology 69,* 814-822.

Luebke, H. J., J. M. Scriber and B. S. Yandell (1988): Use of multivariate discriminant analysis of male wing morphometrics to delineate a hybrid zone for *Papilio glaucus glaucus* and *P. g. canadensis* in Wisconsin. *Amer. Midl. Nat. 119,* 366-379.

Mayr, E. (1963): *Animal Species and Evolution.* Harvard Univ. Press, Cambridge.

Moore, W. S. (1977): An evaluation of narrow hybrid zones in vertebrates. *Q. Rev. Biol. 52,* 263-278.

Opler, P. A. and G. O. Krizek (1984): *Butterflies East of the Great Plains: An Illustrated Natural History.* Johns Hopkins University Press, Baltimore.

Platt, A. P. (1983): Evolution of North American admiral butterflies. *Bull. Entomol. Soc. Amer. 29,* 10-22.

Platt, A. P., S. J. Harrison and T. F. Williams (1984): Absence of differential mate selection in the North American tiger swallowtail, *Papilio glaucus.* In: R. I. Vane-Wright and P. R. Ackery (eds), *The Biology of Butterflies.* Academic Press, New York. 245-250.

Remington, C. L. (1968): Suture-zones of hybrid interaction between recently joined biotas. *Evol. Biol. 2,* 321-428.

Ritland, D. B. and J. M. Scriber (1985): Larval developmental rates of three putative subspecies of tiger swallowtail butterflies, *Papilio glaucus,* and their hybrids in relation to temperature. *Oecologia 65,* 185-193.

Rockey, S. J., J. H. Hainze and J. M. Scriber (1987a): Evidence of a sex linked diapause response in *Papilio glaucus* subspecies and their hybrids. *Physiol. Entomol. 12,* 181-184.

Rockey, S. J., J. H. Hainze and J. M. Scriber (1987b): A latitudinal and obligatory diapause response in three subspecies of the eastern tiger swallowtail, *Papilio glaucus* (Lepidoptera: Papilionidae). *Amer. Midl. Nat. 118,* 162-168.

Scriber, J. M. (1986): Origins of the regional feeding abilities in the tiger swallowtail butterfly: ecological monophagy and the *Papilio glaucus australis* subspecies in Florida. *Oecologia 71,* 94-103.

Scriber, J. M: (1988): Tale of the tiger: Beringial biogeography, binomial classification, and breakfast choices in the *Papilio glaucus* complex of butterflies. In: K. C. Spencer (ed), *Chemical Mediation of Coevolution.* Academic Press, New York. 241-301.

Scriber, J. M., M. H. Evans and D. Ritland (1987): Hybridization as a causal mechanism of mixed color broods and unusual color morphs of female offspring in the eastern tiger swallowtail butterflies, *Papilio glaucus.* In: M. Huettel (ed), *Evolutionary Genetics of Invertebrate Behavior.* Plenum Publ., New York. 119-134.

Scriber, J. M. and J. H. Hainze (1987): Geographic invasion and abundance as facilitated by different host-plant utilization abilities. In: P. Barbosa and J. C. Schultz (eds), *Outbreaks: Ecological and Evolutionary Processes*. Academic Press, New York, 433-468.

Scriber, J. M. and R. C. Lederhouse (1983): Temperature as a factor in the development and feeding ecology of tiger swallowtail caterpillars, *Papilio glaucus* (Lepidoptera). *Oikos 40,* 95-102.

Scriber, J. M., R. L. Lindroth and J. Nitao (1989): Differential toxicity of a phenolic glycoside from quaking aspen leaves to *Papilio glaucus* subspecies, their hybrids, and backcrosses. *Decologia* (in press).

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THE ROLE OF EVOLUTION OF THE DOMINANT SPECIES OF SUCCESSION PROCESSES IN CENOZOIC ECOSYSTEMS IN THE FORMATION OF OLIGOPHAGY OF RECENT PHYTOPHAGES

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ABSTRACT

Evolution of succession systems provides a stabilized arena of speciation of consortiums of oligophages in climax ecosystems. Climax as the last stage is determined by constant generic composition of the dominants throughout a long period of geological history. Peculiarities of generic composition of climaxes reflect zonal evolution of succession systems and alter in the period of geological crises only. Evolution of recent oligophages is connected with the dominants of Cenozoic climaxes, e.g. gall makers on the Amentiferae genera (*Populus, Quercus, Ulmus*) that are not related phylogenetically. Different degrees of oligophagy within the limits of the host groups (a-species, b-genus, c-genera groups, e-family) depend on phylogenetic age of hosts and their participation in domineering on different succession levels. Polyphages are predominant on early stages of succession and prefer phylogenetically young taxa. "Adaptive empire" of oligophages on the dominants of climaxes obtains an opportunity of evolution owing to evolution of succession stages as an integral system directed towards regulation of ecosystems and expanding of ecological niches on the basis of specialization. The succession systems affect and direct phylogenesis. Quantum evolution of phytophages in the Cenozoic is connected with appearing of new climaxes in the Eocene-Miocene. The subsequent formation of oligophagy in stationary ecosystems denies the possibility of phylogenetic "host-parasite" parallelism, because divergence of host is not followed strickly by divergence of parasites.

Key words: evolution, dominant, succession, oligophagy, phytophagy, climax.

INTRODUCTION

Climax and preclimax are the most prolongated and stable stages of succession. And as for climax, it differs from other stages by the balance of produced and decomposed organic substance. This balance determined the stabilization of the terminal stage and because of it, climax has no internal causes for changing. A certain order of succession sequence of communities is characteristic of each particular macroclimate. Only in the periods of global ecological crises and destruction of climax communities conditions for macro-evolution of new links of producers and consumers are created.

Regularities of evolution of oligophagy have been established (Kovalev, 1971, 1988) with reference to co-evolution of gall-maker insects and mites developing on plants of generic taxa predominant for a long period of geological history. The

unusual appearance of specific species of arthropods and fungi on the phylogenetically unrelated taxa of Amentiferae: *Populus*, *Salix*, *Quercus*, *Ulmus*, *Acer* and other is well known. Of 4000 species of gall-maker insects of the Holarctic Region 900 have been described on species of *Salix* and *Populus*, almost half of them on Fagaceae, mainly on *Quercus*. Amentiferae were predominant in the Turgai flora of the Holarctic in the Cenozoic. After the above flora disappeared, they continued to play the predominant role in the recent climax assemblages. Oligophages are predominant on the dominant subtropic Tethys and then on the Mediterranean (Poltava) flora, for instance on Lauraceae and Fagaceae. A similar concentration of oligophages and also of their specific parasites and inquilines is characteristic of genera that were predominant in the tropical zone of the Cenozoic. An opposite picture is known for early stages of vegetation succession, predominance of polyphages among consumers and their preference to phylogenetically young plant taxa. The tendency to prefer food of the predominant species of climax and subclimax ecosystems is typical also of polyphagous vertebrates (mammals, birds).

Polyphages occupy the adaptative zone provided by newly appeared hosts and intense species formation, divergence and reproductive isolation occur simultaneously with host specialization, and then gradual evolutionary and genetic changes follow in the population. The rates of these processes are high at the initial stage of colonizing a new zone, but later they are reduced. Both allogenesis and phyletic evolution occur within the adaptative zone of dominants in climax and subclimax ecosystems.

Quantum outbreak arises in successive food chains following chain reaction. Changes in dominant organisms during geological ages result in arising large taxonomic categories of consumers including tribes, subfamilies, families, orders in the process of quantum evolution. The most complex picture is formed around long available dominant organisms which are in the forefront of evolution.

A long-term predominance of prevailing food species within a community produces an ideal background for evolutionary morphogenesis of the whole specific system in the consortium formed by a dominant in climax ecosystems. The dominant as adaptative zone provides optimal conditions for this. Therefore, consortia of dominants provide the most voluminous evolutionary material for investigations on the origin of adaptations, that is one of the most difficult evolutionary problems. Host predominance is a reflection of a leading evolutionary factor, the "life waves" (Timofeeff-Ressovsky et al., 1969) or population waves in changing sharply the concentration of mutations. The population waves play the most significant role among other main factors including mutations, separation and natural selection. However, "life waves" are mostly short-term fluctuations in population abundance. A dominant host of the stabilized arena of a climax determines fluctuation and mutation concentrations, by this mean it provides a selection background.

The population waves are of no importance for the arising of mutations themselves during elementary evolution, they provide only an evolutionary material, a long-term host predominance is, however, by itself an evolutionary background to any oligophage. While developing on a dominant host, this part of oligophagous population stands a better chance of avoiding an elimination as compared with feeding on a rare

sporadic food species. It should be noted that genotypes quantitatively prevailing in natural populations, show an increase in relative viability (Timofeeff-Ressovsky et al., 1969). Furthermore, predominant food species are distributed over a wide area, that is by itself, a reason of continuous fluctuations in oligophagous populations, while stimulating mutation concentrations a sharp segregation of populations of a dominant in various part of its distribution area, diverse environmental and landscape peculiarities of the populations provide the optimum conditions to organisms and to their geographical morphogenesis.

THE SUCCESSION SYSTEMS AFFECT AND DIRECT PHYLOGENESIS

The influence of ecological factors on the success of animal and plant species is determined mainly by the adaptations to the environments, which stabilized the succession system. The "adaptive empire" of oligophages on the dominants obtains an opportunity of evolution owing to the evolution of succession stages as an integral system directed towards the regulation of ecosystems. It was as early as 1969 when Krasilov proposed that the conditions of ecosystems may determine mutation rate. This idea has been developed by Zherikhin (1987) who supposed that cenotic regulation of evolutionary processes is performed mainly or even exclusively not on the organismic level but on the populational one. We assume that phylogenesis of herbivores is affected and directed by succession systems. Succession systems exist under conditions of a constant macroclimate for a long period of geological history invariably preserving the constant composition of dominant species of climax or subclimax stages. The sequence of series in succession systems changes direction of selection from the initial towards the final ones extending variety of ecological niches at increased specialization and speciation. In the period of geological crises and changes of macroclimate coenophobes become predominant, having descended from species of the early succession stages. Macroevolution is completed by formation of new succession systems, however, a number of arboreal dominants on generic level may enter new climax communities.

Body sizes of the animals determine their relationship to different succession stages. Organisms of small sizes including most of insects, preserve their ecological niches even under considerable changes of the environment. This partly accounts for the slow actual rate of their evolution as compared to the potential one. Among the small organisms most numerous are strict oligophages whose evolution depend directly on the evolution of the succession systems. Different degrees of oligophagy within the limits of the host taxa (a-species, b-genus, c-genera groups, e-family) depend on the phylogenetic age of hosts and their dominance in different succession levels. Evolution of large animals is less dependent on the stage of succession system. For instance vertebrates occur in all forest stages of succession series. Therefore there is a sharp increase in the rate of morphological evolution of terrestrial vertebrates in comparison to the low evolution rate of insects.

Extinction of large animals in terrestrial ecosystems, for example a total disappearing of the "mammoth" fauna, does not affect structural peculiarities of succession links. However, in marine succession systems elimination of large animals leads to destruction of the succession series.

IS THERE REALLY A PHYLOGENETIC PARALLELISM?

The subsequent formation of oligophagy in stationary ecosystems denies the possibility of phylogenetic "host-parasite" parallelism because divergence of hosts is not followed strictly by divergence of parasites.

REFERENCES

Kovalev, O. V. (1971): Host dominance as an evolution of specific parasites. *Proc. XIII. Int. Congr. Entomol., Leningrad 1:* 260-261.

Kovalev, O. V. (1988): Dominants of climax communities in the Cenozoic as an arena of formation of specific oligophages among invertebrates. Nauka Publishers, Moscow. 111-112 (In Russian).

Krasilov, V. A. (1969): Phylogeny and systematics. In: *Problems of Phylogeny and Systematics.* Proceedings of the Symposium, Vladivostok. 12-30.

Timofeeff-Ressovsky, N. W., N. N. Vorontsov and A. V. Jablokov (1969): *An Outline of Evolutionary Concepts.* Nauka Publ., Moscow. (In Russian).

Zherikhin, V. V. (1987): Biocoenotic regulation of evolution. *Paleontol. Zhurn. 1,* 3-12. (In Russian).

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"COEVOLUTION" BETWEEN PARSNIP WEBWORMS AND WILD PARSNIPS: SHOULD THE "CO" GO?

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ABSTRACT

Theories of chemical coevolution between plants and insects have recently been subject to considerable criticism, based on perceived inequities in frequency, impact, and time schedules of interactions. Much of this criticism, however, confounds microevolutionary processes with macroevolutionary patterns. Moreover, many of the proposed tests of theory do not effectively exclude alternative hypotheses. Associations between oecophorid caterpillars and their hostplants will be evaluated at the population level, for evidence of reciprocal selection and genetically based tradeoffs in herbivore performance and plant defense consistent with "microcoevolutionary" processes.

Key words: evolution, coevolution, microevolution, macroevolution, microcoevolution, furanocoumarins, reproduction, parsnip, *Depressaria pastinacella*, *Heracleum*, selection

In Jacobellis vs. Ohio 1964 (378 US 184), a landmark decision involving the nature of obscenity, Supreme Court justice Potter Stewart, unable to provide an objective definition of what he considered "may be undefinable", nonetheless remarked with conviction that "I know it when I see it". In at least one sense, then, obscenity is a lot like coevolution. The term itself, first appearing in the literature over 30 years ago (Mode, 1958), has been used in a dizzying number of contexts, ranging from orthodox descriptions of reciprocal genetic interactions between parasitic fungi and hostplants (the context in which it was first defined) to rather unorthodox applications as in the context of molecular cytogenetics (Gillings et al., 1987) and global climatology (Schneider, 1984). It's no wonder, then, that there has been growing disaffection with the utility of the term and the concepts it represents (Janzen, 1980; Jermy, 1976, 1984; Bernays and Graham, 1988).

However, the problem with an outright rejection of coevolution as a phenomenon is that it is often not clear exactly which meaning of the term is under fire. Many criticisms of coevolution confound two distinct and easily separable processes — microevolutionary phenomena, involving taxa at the species level or above (Brooks, 1988). Parallel cladogenesis (sensu Mitter and Brooks, 1983) is not necessarily the logical result of reciprocal changes in gene frequencies in populations (Janzen, 1980). The same disjunction between micro- and macro-evolutionary processes also might exist between micro- and macro-evolutionary processes. Hypotheses and predictions are different at micro and macro levels, as are the methodologies involved in testing

these hypotheses. Even Ehrlich and Raven (1964), in their classic paper, were reluctant to make specific phylogenetic predictions based on the coevolutionary scenario they proposed.

Jermy (1984) summarized the main premises of what he referred to as "classic coevolutionary theory:

1) Phytophagous insects reduce plant fitness, thus they are important selection factors in plant evolution.

2) Insect attacks select for resistance in plants. This may result in radiation (cladogenesis) of plants.

3) The most important defense mechanisms that have evolved against insects are the secondary plant substances.

4) Interspecific competition is an important factor in insect evolution: specific feeding habits represent "refuges" from competition or "exiles" to which the insects had been confined by the evolution of plant defense mechanisms". Aside from the prediction of cladogenesis, these criteria are essentially populational phenomena, amenable to study with methodologies of quantitative genetics.

There are ample reasons to doubt that, even at the level of populations, coevolutionary interactions occur between insects and plants.

Asymmetries in generation time, frequency of interaction, and selective impact suggest that reciprocity is unlikely in many systems (Berenbaum, 1990). However, the fact that theoretical asymmetries exist is not proof that reciprocal genetic interactions do not, nor is the fact that some systems do not display reciprocity. Systems in which reciprocity may be expected *a priori* are therefore those in which generation time, frequency of interaction, and selective impact are roughly equivalent for plant and insect.

One such interaction is that between *Depressaria pastinacella*, the parsnip webworm, and its hostplant *Pastinaca sativa*, the wild parsnip. The parsnip webworm is oligophagous on *Pastinaca* and the related genus *Heracleum* and occurs throughout the range of its hosts, including in areas of introduction, as in the U. S. Overwintering adults enter reproductive condition in early spring; females oviposit on the overwintering rosettes of their biennial parsnip hosts and emerging larvae feed briefly on leaves and umbel sheaths until the appearance of the flowering stalk, to which they move in order to web together and consume buds, flowers and developing seeds. After larval development is completed, pupation takes place inside the stems of the flowering stalk (Gorder and Mertins, 1984; pers. obs.). Whereas cultivated parsnips have been planted in North America for over 350 years, there are no records of the existence of the parsnip webworm prior to 1869, when it was recorded as a pest on parsnip in Ontario (Bethune, 1869); since that time, it has become established throughout the northern United States and Canada (Clarke, 1952). In parts of its range, the parsnip webworm is effectively monophagous on wild or cultivated parsnip. In that *P. sativa* is a biennial (Baskin and Baskin, 1979) and *D. pastinacella* univoltine (Gorder and Mertins, 1984), generation times are roughly equivalent.

Since its first appearance on the continent, parsnip webworms have had a visible impact on their hosts. As flower, fruit and seed feeders, they are capable of significant

reductions in the production of viable seed on individual plants. Bethune (1869) recounted: "There was a grand prospect of a fine crop of [parsnip] seed, and we began to promise supplies of it to some of our neighbors... all, indeed, looked fair and promising till the last week in June, when 'a change came o'er the spirit of our dream!'. The fine umbels of flowers began to look rather unhappy. Decidedly *seedy* in one sense, but by no means 'seedy' in another. Webs appeared over them, tiny caterpillars were seen to be thick about them, and very soon the big umbels were contracted into shapeless masses of web and excrement, the flowers were all eaten up, the prospect of seed was utterly and entirely gone!". In a more quantitative vein, Thompson (1977) documented much the same phenomenon.

Jermy (1984) argues that interactions between introduced weeds and introduced insects cannot be cited as examples of the ability of insects in general to reduce plant fitness, in part because the insects lack their natural enemies in the new environment and in part because weeds tend to occupy disturbed environments and thus provide insects with an unnaturally concentrated resource. However, evolution does not end with an introduction; introduced populations are capable of responding to selection pressures and to undergoing evolutionary changes in areas of introduction. It is as legitimate to examine introduced species 100 generations after introduction into a new continent as it is to examine interactions of uncertain age in ancestral areas. In fact, in view of the predilection of parsnips for marginal habitats, any single population even in the areas of indigeneity may maintain its integrity only for a few years at most.

In the case of the parsnip webworm/wild parsnip interaction, there is quantitative genetic evidence of a selective impact of the insects on plant populations. Plant families do indeed experience differential reproductive success in the presence of webworms (Berenbaum et al., 1986). Although wild parsnip has been shown to display reproductive compensation to some extent to webworm damage, such compensation is incomplete (Hendrix and Trapp, 1989) and is effectively eliminated in heavy infestations in which massive pupal excavation of the stem results in total collapse of the plant (personal observation).

Differential reproductive success, however, does not necessarily imply that "insect attacks select for resistance in plants", Jermy's second criterion. For parsnip webworms and wild parsnips, there is quantitative genetic evidence of selection for increased resistance as a result of insect attack. Furanocoumarins are broadly biocidal and are thus likely resistance factors in wild parsnip in particular and umbelliferous plants in general (Berenbaum, 1990). Stepwise regression analysis revealed that amounts of two furanocoumarins, bergapten and sphondin, in seeds are associated with resistance to parsnip webworm damage in the population studied. Halb-sib heritability estimates of these traits are significantly different from zero, indicating that there is significant additive genetic variance in these traits upon which selection can act. Estimates of selection differentials indicate that at least one of these traits, the proportion of bergapten in the seeds, undergoes a change in distribution in the plant population as a result of the pattern of insect attack (Berenbaum et al., 1986).

Table 1 First instar mortality (%) of *Depressaria pastinacella* from two locations on seeds of *Pastinaca sativa* from the same locations (sample sizes in parentheses).

	Insect Population			
	Phillips Tract	Phillips Tract	Race Street	Race Street
Plant population	Phillips Tract	Race Street	Phillips Tract	Race Street
Insect mortality	6.7/(2/30)*	30.0 (9/30)*	20.0 (6/30)	8.7 (2/23)

*Significantly different at $p<0.05$ (G test)

Pupae collected summer 1985 and reared through adulthood in the laboratory: seeds were administered to neonates as 3% wet weight of artificial diet (Nitao and Berenbaum, 1988) by J. K. Nitao.

A comparison of two wild parsnip populations, subject to different levels of webworm infestation, revealed heritable differences in furanocoumarin chemistry consistent with selection for resistance. In one population, with evidence of insect attack on every plant sampled and with webworm densities in excess of 11 pupae per stem, constitutive foliar furanocoumarin content of bergapten was significantly higher than in a population approximately 10 km distant with a frequency of infestation only 50% as high and with substantially lower webworm densities per stem; heritabilities of both constitutive and induced furanocoumarin content of foliage indicate that additive genetic variance is available for selection on these traits (Zangerl and Berenbaum, submitted).

Coevolution at the population level (Janzen, 1980) implies reciprocal genetic changes in both plant and insect populations; there is at least preliminary evidence that webworm populations are subject to selection by their hostplants. Reciprocal rearing of webworms from two populations on seeds from their home population and from the "foreign" population results in significantly greater survival of webworms on seeds from the home population (Table 1).

That the differences in furanocoumarin chemistry associated with resistance in wild parsnip (Berenbaum et al., 1986) are in fact responsible for differences in survival of parsnip webworms is indicated by direct bioassay and by metabolism studies. Whereas webworms are capable of metabolizing xanthotoxin via cytochrome P450 monooxygenases extremely efficiently (Nitao, 1989), bergapten and sphondin are metabolized at significantly lower rates than is xanthotoxin. When incorporated into artificial diets bergapten, caused a significant reduction in relative growth rate, approximate digestibility, and relative consumption rate (Berenbaum et al., 1989). Since pupal weight, and relative growth rate are traits with measurable additive genetic variance in parsnip webworms (half-sib heritability estimates 0.15 and 0.13 respectively), bergapten concentrations can potentially act as selective agents on webworm populations.

Within the subfamily Depressariinae, to which *Depressaria* belongs, flower and fruit feeding are restricted to only a few genera; even within the genus *Depressaria*, flower and fruit feeding are found in variable degrees (Thompson, 1983). Parsnip webworms are incapable of surviving on foliage of wild parsnip alone (personal observation). Jermy (1984) suggests that the contention that specific feeding habits

are "'exiles' to which... insects had been confined by the evolution of plant defense mechanisms" is untenable due to the absence of interspecific competition among herbivorous insects. This contention, alone of all of Jermy's criteria, cannot really be tested outside the area of indigeneity of parsnip webworms. In Europe, where *Depressaria pastinacella* is native, several congeners feed on *Pastinaca, Heracleum,* and other hosts of *D. pastinacella*; these include *D. badiella*, in the same species group as *D. pastinacella*, and *D. gudmanni*, in the *douglasella* group (Hanneman, 1953). An examination of the *D. pastinacella* group in Europe would prove instructive regarding the origin of the feeding habits of this species. Interspecific competition may prove extremely subtle in this complex of species inasmuch as *D. pastinacella* larvae are capable of consistently discriminating among seeds of different plant genotypes — and even among seeds of different viability within an umbel — in laboratory bioassays (Zangherl and Berenbaum, submitted). Whereas individual wild parsnip plants may not be in short supply in an area, suitable or preferred genotypes may be; virtually no studies of interspecific competition among species sharing a hostplant *species* take into account potential differences in suitability of different *genotypes* within hostplant populations.

In conclusion, in order to argue the existence or nonexistence of a phenomenon called "coevolution", it is first and foremost necessary, if not to define it, then at least to agree on characters by which it can be identified. Three major sources of error can thereby be eliminated — rejection of a potentially widespread phenomenon based on a specific and possibly inappropriate example, denial of relativistic or comparative results on absolute criteria, and dismissal of a putative microevolutionary process based on a macroevolutionary pattern. While evaluating the validity of coevolutionary theory may not involve the same set of rules as evaluating obscene or pornographic material, there are challenges common to both enterprises.

REFERENCES

Baskin J. M. and C. M.. Baskin (1979): Studies on the autecology and population biology of the weedy monocarpic perennial *Pastinaca sativa*. *J. Ecol. 67,* 601-610.

Berenbaum, M. (1981): Patterns of furanocoumarin production and insect herbivory in a population of wild parsnip (*Pastinaca sativa* L.). *Oecologia 49,* 236-244.

Berenbaum, M. R. (1990): Evolution of specialization in insect-umbellifer associations. *Ann. Rev. Entomol. 35,* 319-343.

Berenbaum, M. R., A. R. Zangerl and J. K. Nitao (1986): Constraints on chemical coevolution: wild parsnip and the parsnip webworm. *Evolution 40,* 1215-1228.

Berenbaum, M. R., A. R. Zangerl and K. Lee (1989): Chemical barriers to adaptation by a specialist herbivore. *Oecologia 80*, 501-506.

Bernays, E. and M. Graham (1988): On the evolution of host specificity in phytophagous arthropods. *Ecology 69,* 886-892.

Bethune, C. J. S. (1869): Larva infesting the parsnip (*Depressaria ontariella* n. sp.). *Can. Entomol. 2,* 1-4.

Brooks, D. R. (1988): Macroevolutionary comparisons of host and parasite phylogenies. *Ann. Rev. Ecol. Syst. 19,* 235-259.

Clarke J. F. G. (1952): Host relationships of moths of the genera *Depressaria* and *Agonopterix*, with descriptions of new species. *Smithson. Misc. Coll. 117,* 1-20.

Ehrlich, P. R. and P. H. Raven (1964): Butterflies and plants: a study in coevolution. *Evolution 18,* 586-608.

Gorder, N. K. N. and J. W. Mertins (1984): Life history of the parsnip webworm, *Depressaria pastinacella* (Lepidoptera: Oecophoridae) in central Iowa. *Ann. Entomol. Soc. Amer. 77,* 568-573.

Gillings, M. R., R. Frankham, J. Speirs and M. Whalley (1987): X-Y exchange and the coevolution of the X and Y rDNA arrays in *Drosophila melanogaster. Genetics 116,* 241-251.

Hannemann, H. J. (1953): Natürliche Gruppierung der Europäischen Arten der Gattung *Depressaria* s. l. (Lep. Oecoph.). *Mitt. Zool. Mus. Berlin 29,* 269-373.

Hendrix, S. D. and E. J. Trapp (1989): Floral herbivory in *Pastinaca sativa*: do compensatory responses offset reductions in fitness? *Evolution 43,* 891-895.

Janzen. D. H. (1980): When is it coevolution? *Evolution 34,* 611-612.

Jermy, T. (1976): Insect-host-plant relationships — coevolution or sequential evolution? *Symp. Biol. Hung. 16,* 109-113.

Jermy, T. (1984): Evolution of insect/host-plant relationships. *Am. Nat. 124,* 609-630.

Mitter, C. and D. Brooks (1983): Phylogenetic aspects of coevolution. In: D. Futuyma and M. Slatkin (eds), *Coevolution.* Sinauer Associates, Sunderland. 65-98.

Mode, C. J. (1958): A mathematical model for the co-evolution of obligate parasites and their hosts. *Evolution 12,* 158-165.

Nitao, J. K. (1989): Enzymatic adaptation in a specialist herbivore for feeding on furanocoumarin-containing plants. *Ecology* (in press)

Nitao, J. K. and M.R. Berenbaum (1988): Laboratory rearing of the parsnip webworm, *Depressaria pastinacella* (Lepidoptera: Oecophoridae). *Ann. Entomol. Soc. Amer. 81,* 485-487.

Schneider, S. H. L. (1984): *The Coevolution of Climate and Life*. Sierra Books, San Francisco.

Thompson, J. N. (1977): Within-patch structure and dynamics in *Pastinaca sativa* and resource availability to a specialized herbivore. *Ecology 59,* 443-448.

Thompson, J. N. (1983): Selection of plant parts by *Depressaria multifidae* (Lep. Oecophoridae) on its seasonally-restricted hostplant, *Lomatium grayi* (Umbelliferae). *Ecol. Entomol. 8,* 203-211.

Zangerl, A. R. and M. R. Berenbaum: Furanocoumarin induction in wild parsnip: genetics and populational variation. Submitted to *Ecology.*

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HERBIVORY AND THE EVOLUTION OF THE ANGIOSPERMS

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ABSTRACT

The adaptive radiation of the angiosperms is viewed as an evolutionary response to herbivores. Many angiosperms seem to have evolved defences against herbivory but it is possible that mutualistic evolution is also involved. One experiment demonstrates that herbivory enhances plant fitness and the results are strongly suggestive of a mutualistic association.

Key words: angiosperm, mutualism, fitness, evolution, herbivory, grasses.

INTRODUCTION

The question of where the angiosperms came from and the sequence of evolution of major lines has been repeatedly examined and reviewed (e.g. Doyle, 1978). It seems agreed that angiosperms did not 'replace' gymnosperms during their main radiation in the Cretaceous, and the situation is evidently not analogous to the replacement of dinosaurs and their relatives by mammals. Fossil and anatomical evidence provide much information on angiosperm evolutionary pathways, but little indication of the nature of the selective forces responsible for the radiation. Climatic change is frequently invoked as the stimulus for angiosperm radiation, but then climatic change is always invoked to explain ecological and evolutionary phenomena when no one can think of other explanations.

Major radiations of insects and other plant-feeding animals also occurred in the Cretaceous. Few dispute the mutualistic evolution of flowers and their pollinators, and many (but not all) accept a mutualistic evolution between fruits and fruit-eaters. On the other hand foliage eating (in the widest sense which includes chewing and sucking of living plant tissue) is commonly viewed as a matter of attack and defence: herbivores feed on foliage and the evolutionary response of plants is the development of a battery of chemical and physical defences which are clearly apparent to us all. Another view is that the chemical and physical diversity of angiosperms preceded their exploitation by herbivores and that the evolution of the two groups is 'sequential' (Jermy, 1984).

I favour the view that the evolution of angiosperms and herbivores is a reciprocal process, the one not being possible without the other, although it is by no means easy from an examination ot the contemporary scene to decide whether a given plant and plant-eater are really co-evolved. I also favour the view that this reciprocal process is not necessarily always a matter of attack and defence: reciprocal mutualistic adaptive radiation is an alternative explanation.

In 1976 we asked a question, 'Do consumers maximise plant fitness?' and offered evidence suggesting that under certain circumstances the answer might be 'yes' (Owen and Wiegert, 1976). The question stimulated a long-running debate in the Forum section of Oikos, most (but not all) contributors arguing that the answer is 'no'. Nevertheless an uneasy feeling developed that the widely held attack and defence approach might not always be appropriate to explain many of the adaptive features of plants which in one way or another seem associated with herbivory. In a later contribution (Owen and Wiegert, 1981) we focused on grazers and grasses as it seemed to us that here was the best evidence for mutualistic evolution.

FITNESS AND MUTUALISM

I acknowledge the difficulty of defining, let alone measuring, relative fitness in mutualistic associations. Fitness is the contribution of offspring of one genotype relative to the contribution of others, and I agree with Williams (1966) that whether for alleles or individuals, fitness has no meaning unless we know how others are doing. For plants fitness could comprise two components: one associated with immediate reproduction or prospects of reproduction, the other associated with longer-term survival to achieve future reproduction and favour ramet production, but may prolong the life of the individual to an extent that it eventually achieves a higher fitness than an ungrazed plant.

In the context of herbivore/plant relationships, mutualism is the association of two unrelated organisms (normally an animal and a plant) such that the relative fitness of each is higher than it would be if they existed by themselves. Any tendency on the part of one of the pair to form a more beneficial (in terms of fitness) relationship results in the evolution of traits in the other which facilitate the advantage of the new tendency.

There has been remarkable reluctance to recognize and study possible herbivore/plant mutualisms, but there has been (and still is) an equally remarkable willingness to adopt the language of attack and defence. Plants are credited with a wide array of anti-herbivore defensive adaptations, but even though rather few examples have been experimentally verified, the attack and defence argument is all pervasive. I suggest that the mere presence in plants of compounds and structures that are unrelated to normal growth and development does not necessarily imply a defensive function, and yet once such attributes are dubbed defensive questions are asked and experiments performed to elucidate the nature of the defensiveness.

The use of the word 'predator' to describe a plant-eater can slant the interpretation of what really happens when an animal consumes part of a plant. True, modules or other plant parts are eaten, but the genetic individual is rarely killed. Modules may be capable of reproduction but they are not complete genetic individuals, and are hence not important in evolution by natural selection. Nevertheless grazing and browsing are often described in terms of a predator/prey relationship.

OBSERVATIONS AND PROPOSITIONS

Two observations are fundamental to the concept of mutualism between herbivores and angiosperms. First, land plants are usually eaten without being killed; indeed a common response to browsing and grazing is vigorous regrowth. Secondly, the level of primary consumption in terrestrial ecosystems is low and most of the plant material produced is decomposed once it is dead. Neither of these observations apply in a pelagic system where the plants are small plankton and are eaten whole (here the word 'predator' is more apt) and where the level of consumption of living individuals is high, often over 90% (Wiegert and Owen, 1971). The low level of consumption of living plant material in terrestrial ecosystems had earlier been noted by Hairston et al. (1960) who suggested that primary consumers (as a trophic level) are not food-limited and therefore must be predator and parasitoid-limited. This conclusion generated much discussion as to whether or not there is interspecific competition between herbivores (especially insect herbivores) which, in the event, is not essential to the question of mutualistic evolution between herbivores and angiosperms.

The idea that angiosperms and their consumers are mutualistically evolved may be hard to accept and there will undoubtedly be much further argument and discussion. But consider the following propositions:

1. The ability to photosynthesize is of limited value to a plant if growth and subsequent reproduction are inhibited by a scarcity of nutrients. Plants, it seems, are more often restricted by the availibility of nutrients than by the acquisition of radiant energy.

2. Hence, natural selection should favour traits that increase the rate of supply of essential nutrient.

3. There are severe restrictions to a plant's ability to release its own 'excess' products of photosynthesis, but by enlisting the 'help' of a consumer there are better prospects. If the consumer excretes sugars or other substances from the plant it feeds on, or if it causes premature or unseasonal shedding of leaves, these may be mobilised beneath the individual plant from which they came and subsequently make available nutrients in short supply. Thus excess sugar produced by rampant photosynthesis and released from plants by aphids may provide a resource for free-living nitrogen-fixing bacteria, and hence enhance the rate of supply of nitrogen to the plant from which the sugar originated. It has to be admitted that attempts to demonstrate this

possibility have produced conflicting results partly, I suspect, because of differences in the various techniques used in assessing nitrogen fixation.

4. A herbivore may affect the growth form of the plant especially if, as is often the case, growing points are consumed which then induces multiple lateral branching or several inflorescence-bearing shoots in the place of one. This could result in a better structured plant with the photosynthetic parts better positioned and more seed from the increased number of branches or shoots. A recent experiment demonstrates that when the biennial, *Ipomopsis aggregata* (Polemoniaceae), is browsed by deer and elk, the plant responds by producing multiple inflorescences and up to three times as many flowers, fruits and seeds as an unbrowsed plant (Paige and Whitham, 1987).

5. The possibility of mutualism between grasses and grazers is attractive but it is fraught with experimental difficulty because almost everywhere grasslands are heavily grazed by cows, sheep and goats, products of man's ingenuity. Indeed it is hard to find truly natural grassland anywhere in the world. Even in reserves and national parks there tends to be overgrazing as the grazing mammals exist at artificially high densities. To a large extent the impact of grazing insects, such as Orthoptera, is obscured by the effects of man's domestic stock.

The grass family, with about 7,500 species, first appeared just before the Miocene and is therefore relatively recent in the history of angiosperm evolution. Levels of consumption by grazers tend to be high, which suggests greater palatability than in other terrestrial plants. Certain species of grasses occur only in association with specific grazers, as on the Aldabra atoll where three endemic species are found only where there is grazing by giant tortoises. This kind of association may have been more widespread and common before the enormous impact of domesticated grazers commenced about ten thousand years ago.

Grasses have a basal meristem, too low to be destroyed by most grazers, and after being grazed leaves and stems quickly regrow; the formation of numerous ramets is also stimulated. Most perennial grasses reproduce vegetatively and very old and very large genets are formed; indeed the genet (the evolutionary individual) is potentially immortal especially, it seems, if subjected to periodic grazing.

Of course, all the above features could have evolved as anti-grazing adaptations, but is it not equally likely that the evolution and growth form of grasses is the result of mutualism? Experimental evidence is difficult to assess and it might be that the correct experimental design has not yet been formulated. One problem is that grass ecologists seem preoccupied with measurements of productivity (biomass estimates) rather than with seed production (a better method of assessing evolutionary fitness).

CONCLUSION

Belsky (1986) in a review of the evidence that herbivory benefits plants concludes that the idea lacks hard evidence in the form of properly designed experiments. She was probably right. But it seems to me that the work of Paige and Whitham (1987)

is the first unequivocal piece of experimental evidence that consumers can maximize plant fitness and that mutualism is involved in herbivore/angiosperm relationships.

There is indeed a conspicuous lack of evidence based on properly designed experiments with clear-cut, statistically significant results. Such experiments are fine ideals, but if the need for them were universally applied to the study of the evolutionary process there would be rather little to say. Thus although it is accepted that natural selection is the driving force of evolution, hard evidence is scarce and is usually confined to demonstrating changes in gene frequency at the population level.

There is another approach which is decidedly non-experimental. It comprises making observations on structure, function and ecology; it relies heavily on how the observations are interpreted; and above all it is careful about the use of words. Charles Darwin referred to the evolution of higher plants as an 'abominable mystery' (F. Darwin, 1905), and it will remain a mystery unless current dogma are from time to time challenged with ideas which, although unconventional, may provide a key to a new look at herbivore/plant interactions. Certainly, most still view herbivore/plant relationships as a matter of attack and defence, but this does not necessarily mean this viewpoint is correct, and so I repeat the question we put in 1976, 'Do consumers maximize plant fitness?' It is after all only a question.

REFERENCES

Belsky, A. J. (1986): Does herbivory benefit plants? A review of the evidence. *Am. Nat. 127*, 870-892.

Darwin, F. (1905): *More Letters of Charles Darwin* Vol. 2, Murray, London.

Doyle, J. A. (1978): Origin of angiosperms. *Ann. Rev. Ecol. Syst. 9*, 365-392.

Hairston, N. G., F. E. Smith and L. B. Slobodkin (1960): Community structure, population control, and competition. *Am. Nat. 94*, 421-425.

Jermy, T. (1984): Evolution of insect/host plant relationships. *Am. Nat. 124*, 609-630.

Owen, D. F. and R. G. Wiegert (1976): Do consumers maximize plant fitness? *Oikos 27*, 488-492.

Owen. D. F. and R. G. Wiegert (1981): Mutualism between grasses and grazers: an evolutionary hypothesis. *Oikos 36*, 376-378.

Paige, K. N. and T. G. Whitham (1987): Overcompensation in response to mammalian herbivory: the advantage of being eaten. *Am. Nat. 129*, 407-416.

Wiegert, R. G. and D. F. Owen (1971): Trophic structure, available resources and population density in terrestrial vs. aquatic ecosystems. *J. Theor. Biol. 30*, 69-81.

Williams, G. C. (1966): *Adaptation and Natural Selection*. Princeton University Press, Princeton.

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CHEMICAL SPECIALIZATION ON TOXIC PLANTS PROVIDES INCREASED PROTECTION FROM NATURAL ENEMIES

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ABSTRACT

Oreina (Chrysomelinae, Coleoptera) is a specious genus of montane leaf-beetles feeding on either Apiaceae or Asteraceae. In most species, the adults synthesize *de novo* cardenolides, which are stored by and subsequently liberated from defensive glands when the insects are disturbed. Two species differ considerably from this pattern: *O. cacaliae* feeding on *Senecio, Adenostyles,* and *Petasites* secretes only pyrrolizide N-oxides which are sequestered directly from their host plants. *O. speciosissima*, a sympatric species feeding on the same plants, uses both defensive strategies: *de novo* synthesis from cardenolides as well as sequestration of plant toxins. This system provides insight into the evolution of sequestration of plant toxins by herbivores. We suggest that this sequestration was historically associated with a change of host plant. We suggest further that this shift of host plant was followed by a change in the reproductive biology of the beetles.

Key words: chemical defense, ovovivipary, reproductive strategy, cardenolides, pyrrolizidine, Chrysomelinae, *Oreina*, Asteraceae.

INTRODUCTION

The adults of the leaf-beetle *Oreina* are colorful and conspicuous. Patchy distribution of their food plants and the low dispersion rate of larvae and gravid females tend to produce large aggregations of leaf beetles forming locally isolated populations at middle (600m) to high (2000m) elevations. Chemical defensive mechanisms are characteristic of the Chrysomelinae. The defensive compounds are not generally distributed throughout the body, rather the beetles are defended by secretion oozing from dorsal glands opening along the lateral margins of the thoracic terga and elytra (Deroe and Pasteels, 1982). The chemical nature of the toxins present in the adults' glands is well known in the European species of the tribe Chrysomelini to which *Oreina* belongs (Pasteels et al., 1988a,b and 1989b).

Among the potential natural enemies of the beetles are insectivorous predators such as shrews living in the same biotope. Indeed, the toxic adults (*O. cacaliae*) are avoided by shrews (*Crocidura* sp. and *Sorex* sp.) when these predators are given an alternative non-toxic prey (grasshoppers or meal worms) (see Table 1). In 20 choice

Table 1 Choice by shrews between non-toxic prey and leaf-beetles (*Oreina cacaliae*).

Naive shrews	eat mealworm	eat beetles	do not eat
20 *Sorex coronatus*	16	0	4
17 *Crocidura russula*	12	1	4

Table 2 Host plants of *Oreina* spp.

O. alpestris	Apiaceae (generalist)
O. speciosa	Apiaceae (generalist)
O. variabilis	Apiaceae (generalist)
O. bifrons	Apiaceae (specialist on *Chaerophyllum*)
O. gloriosa	Apiaceae (specialist on *Peucedanum*)
O. bidentata	Asteraceae (*Centaurea*)
O. rugulosa	Asteraceae (*Centaurea*)
O. tristis	Asteraceae (*Centaurea*)
O. speciosissima	Asteraceae (*Senecio, Adenostyles, Petasites*)
O. cacaliae	Asteraceae (*Senecio, Adenostyles, Petasites*)

tests with individual shrews, 16 ate the control prey, 4 did not feed at all, and none fed on *Oreina*.

Another remarkable feature of this genus is the ovovivipary of most species. This means that the first larval stage develops inside the female for a period that varies between species. The larvae have no exocrine defensive glands of their own.

Oreina is an extremely specious genus of central Europe. The different species are often impossible to tell apart without examining the male genitalia. *Oreina* feeds on Apiaceae and Asteraceae (Jolivet et al., 1986), both families containing numerous toxins (e.g. furanocoumarins, sesquiterpene lactones, alkaloids and others) (Hegnauer, 1986). The food plant preference of 10 species of *Oreina* was established by regular field observations and by laboratory feeding experiments. The results are summarized in Table 2. Three species will be discussed in more detail in this paper. *O. gloriosa* is specialised on *Peucedanum osthruthium* (Apiaceae). *O. speciosissima* and *O. cacaliae* are oligophagous sympatric species feeding on *Adenostyles* spp., *Senecio* spp. and *Petasites* spp., all belonging to the Asteraceae.

Our aim was to investigate both the relationship between the host plants' secondary metabolites and the beetles' chemical defenses as well as the possible link between chemical defense and reproductive biology.

ADULT CHEMICAL DEFENSE

The exocrine defensive secretions of 10 species of *Oreina* were collected and analysed by HPLC and TLC. The genus *Oreina* can be divided in 2 groups of species:

Table 3 Reproductive strategy of different species of *Oreina*

Species (defense)	No. larvae on day 20	mg/larva	mg/adult	Total mg larva/adult
O. gloriosa	25.5	2.36	74.3	0.81
(cardenolide)	(9±11.9)	(36±0.69)	(50±14.39)	
O. speciosissima	40.2+	1.08***	57.2***	0.76
(card.+PA)	(9±17.9)	(32±0.29)	(20±7.4)	
O. cacaliae	45.0**	1.26***	69.9+	0.81
(PA)	(10±11.3)	(34±0.30)	(50±10.8)	

+:0.1>p>0.05; **:p<0.01; ***:p<0.001; where p is the probability of differing from *O. gloriosa*.

those (*O. bidentata, bifrons, gloriosa, rugulosa, speciosa, speciosissima, tristis, variabilis*) secreting cardiac glycosides, which they can synthesize *de novo* like many other species of Chrysomelinae (Van Oycke et al., 1987), and *O. cacaliae* and *O. speciosissima* secreting a completely different type of chemical: pyrrolizidine N-oxides sequestered from their food plants (Pasteels et al., 1988c; Pasteels et al.; 1989a,b).

In collaboration with the group of Prof. Hartmann in Braunschweig we confirmed that the compounds present in the secretion of *O. cacaliae* and *O. speciosissima* are from host plant origin. This was done by feeding the beetles radioactive compounds and recovering the radioactivity in the secretion.

IS REPRODUCTIVE STRATEGY INFLUENCED BY CHEMICAL DEFENCE?

Both in the field and in the laboratory *O. cacaliae* and *O. speciosissima* give birth to their larvae immediately after the eclosion of the latter from the egg, early in the first instar. *O. gloriosa* females, on the other hand, produce larvae which are developmentally more advanced, they are born much later at the end of the first instar.

The theory of parental investment predicts that in species whose larvae spend a longer part of their development *in utero,* and thus benefit longer from the protection afforded by the maternal chemical defense, the number of offspring should be smaller than in species where the larvae benefit less from the maternal protection. We were able to confirm experimentally this theoretical prediction. The number of offspring per female is higher and produced faster in *O. cacaliae* and *O. speciosissima* than in *O. gloriosa* as indicated in Table 3 by the number of larvae produced during the first 20 days of reproduction, after which only a very small number of larvae is produced.

Both the weight of the larvae at birth (mg/larva) and the weight of the females (mg/adult) are smaller in *O. cacaliae* and *O. speciosissima* than in *O. gloriosa*. All three species, however, invest the same proportion of their adult weight (total mg larva/adult) in reproductive effort (Table 3).

The reason why the larvae of *O. cacaliae* and *O. speciosissima* spend less time in their mother may be that they also use plant toxins such as pyrrolizidine alkaloids

Table 4 Observations of ant (*Messor* sp.) behavior (First 12 min. after encounter) when contacting different species of larvae offered in a triple choice experiment (5 repetitions).

Prey spp.	No. ants staying	No. ants leaving
Grasshopper femur	8.6±1.8	0
O. speciosissima (larvae fed without PA)	3.6±2.7	5.6±2.9
O. cacaliae (larvae fed with PA)	1.2±1.67	7.8±1.9

(PAs) for protection, rather than rely on the cardenolides produced by their mother, as do the larvae of *O. gloriosa*. This hypothesis is supported by the fact that the larvae of *O. gloriosa* are cryptic and feed under the leaves, whereas those of *O. speciosissima* and *cacaliae* are aposematic and feed on top of the leaves.

Further, the fact that the larvae might be protected by plant PA is supported by preliminary experiments with ants: the results (Table 4) show that larvae of *O. cacaliae* and of *O. speciosissima* are repellent to ants compared to a grasshopper femur of the same size. Additionally, larvae of *O. cacaliae*, reared on plants rich in PAs, are more repellent than larvae of *O. speciosissima*, reared on plants with only traces of PAs. The larvae of these 2 species are otherwise similar in morphology and coloration. Repellent activity is also found in methanolic extracts of larval exuvia.

CONCLUSIONS

The sequestration of PAs by *O. cacaliae* and *O. speciosissima* is clearly established, it is the first known example of the use of plant secondary compounds by adult Chrysomelinae in their glandular defensive secretion.

Other date on food-plant preferences in *Oreina* spp. and on isozyme genetic distances between them suggest that this sequestration was historically associated with a change of host plant. We suggest further that this shift of host plant was followed by a change in the reproductive biology of the beetles.

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REFERENCES

Deroe, C. and J. M. Pasteels (1982): Distribution of adult defense glands in chrysomelids (Coleoptera: Chrysomelinae) and its significance in the evolution of defense mechanisms within the family. *J. Chem. Ecol. 8*, 67-82.

Hegnauer, R. (1986): *Chemotaxonomie der Pflanzen VII*. Birkhäuser, Basel.

Jolivet, P., E. Petitpierre and M. Daccordi (1986): Les plantes-hôtes des Chrysomelinae. Quelques nouvelles précisions et additions (Coleoptera). *Nouv. revue Entomol. 3*, 341-357.

Pasteel, J. M., J. C. Braekman and D. Daloze (1988a): Chemical defense in the chrysomelinae. In: P. Jolivet and T. H. Hsiao (eds), *The biology of Chrysomelinae*. Junk, Dordrecht, 233-252.

Pasteels, J. M., M. Rowell-Rahier and M. J. Raupp (1988b): Plant derived defense in chrysomelid beetles. In: P. Barbosa and D. Letourneau (eds), *Novel Aspects of Insect-Plant Interactions*. J. Wiley and Sons, New York, 235-272.

Pasteels, J. M., M. Rowell-Rahier, T. Randoux, J. C. Braekman and D. Daloze (1988c): Pyrrolizidine alkaloids of probable host-plant origin in the pronotal and elytral secretion of the leaf beetle *Oreina cacaliae*. *Entomol. exp. appl. 49*, 55-58.

Pasteels, J. M., S. Duffey and M. Rowell-Rahier (1989a): Toxins in Chrysomelid beetles: Possible evolutionary sequence from de novo synthesis to derivation from food plant chemicals. *J. Chem. Ecol.* (in press).

Pasteels, J. M., M. Rowell-Rahier, J. C. Braekman, D. Daloze and S. Duffey (1989b): Evolution of exocrine defense in leaf-beetles (Coleoptera: Chrysomelinae). *Experientia 45*, 295-300.

Van Oycke, S., J. C. Braekman, D. Daloze and J. M. Pasteels (1987): Cardenolides biosynthesis in chrysomelid beetles. *Experientia 43*, 460-462.

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POPULATION STRUCTURE AND EVOLUTION IN SEXUAL AND PARTHENOGENETIC LEAF MINING MOTHS (LEPIDOPTERA, NEPTICULIDAE): WHY SO LITTLE SPECIATION?

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ABSTRACT

Sexual and parthenogenetic species of the genera *Ectoedemia* and *Stigmella* have been studied by allozyme analysis for their population structure. Substructuring within and a large differentiation among populations were revealed, indicating low levels of gene flow. This can be understood from the short life span, egg-laying behaviour, and dispersal abilities of the adult moths. Most populations are far from being genetically impoverished, harbouring amounts of allozyme variation that are large compared with other – non-*Drosophila* – insect species. All in all these are characteristics that render nepticulid species prone to speciation. Yet almost all species pairs (including sibling species) have low genetic identity values. With the exception of some species feeding on *Quercus* no recent speciation seems to have taken place.

Key words: *Ectoedemia*, *Stigmella*, allozymes, gene flow, natural selection, genetic drift, inbreeding coefficients, *Quercus*.

INTRODUCTION

Speciation, the formation of two or more species from a common ancestor, is a critical step in evolution. Without genetic variation evolution does not occur; natural selection for better-adapted genotypes cannot operate and genetic drift does not exist. Yet populations without measurable variation apparently can survive at least in the short run (for an extreme example see Menken, 1987). Genera will contain species at various levels of relationships depending on the time elapsed since a common ancestor. Assuming a molecular clock, genetic differentiation values among congeneric species will span the entire range from almost identical to totally different (see Fig. 2 in Menken and Ulenberg, 1987).

Nepticulidae are a Monotrysian family of very small moths the larvae of which mine mainly in the leaves, but also in the petioles, bark, and seeds of primarily dicotyledon tree species. They have a world-wide distribution and with more than 600 described species are by far the largest family of non-Dytrisian Lepidoptera. In studying the biochemical systematics of the family generally large genetic distances were encountered (Wilkinson et al., 1983; Menken and Brouwer, 1984; Van Driel and Menken, 1988; Cronau and Menken, 1989; Menken, unpublished). This suggests

Table 1 Mean genetic distances (D) in Nepticulidae, n being the number of species

Species pair or species group	n	D (range)
S. betulicola group	6	3.093 (1.396-8.041)
S. lapponica group	2	1.269
S. ruficapitella group	5	0.961 (0.218-2.008)
E. angulifasciella group	4	0.607 (0.450-0.711)
S. aurella-splendidissimella	-	1.556
E. populella group	5	1.063 (0.241-1.637)

that with the exception of some species on oak, no recent speciation has taken place. However, in the present paper preliminary data on population structure are presented which together with life cycle characteristics indicate that speciation in Nepticulidae should be facilitated.

MATERIALS AND METHODS

Sample preparation, electrophoresis, and enzyme assays follow Van Driel and Menken (1988). Specimens were studied at 12-18 genetic loci. Genetic interpretation was inferentially based on the general fit of observed phenotypic proportions to those expected under Hardy-Weinberg equilibrium. Full data will be presented elsewhere.

RESULTS AND DISCUSSION

Table 1 presents genetic distance values between various nepticulid species calculated from allozyme frequencies found (Nei, 1972). Sibling species differ much (e.g., *Stigmella aurella* and *S. splendidissimella*) and species belonging to different species groups (on morphological grounds) within a genus often do not share any allozyme band (not shown). Even in *S. basiguttella* where a totally cryptic species was observed the two taxa were fixed for different alleles at several loci (Cronau and Menken, 1989; Menken, 1989). Some species of the *E. subbimaculella* group (all species are oak-miners) form a notable exception to this rule. For example, the morphologically quite dissimilar species *E. liechtensteini* and *E. heringi* have a genetic distance of only 0.003.

One may ask if nepticulid populations are genetically depauperate which might hamper their evolutionary possibilities. As for allozyme variation as a general means of estimating genetic variability, this is generally not so. In a study of 33 species in the genus *Ectoedemia* an average (±s.d.) heterozygosity (*H*) was found of 0.110±0.054 (range 0.000-0.264). *Stigmella* species cover a similar range. This is high for non-*Drosophila* insect species (Nevo et. al., 1984: *H* values for *Drosphila* [34

species] and non-*Drosophila* [122 species] were 0.123±0.053 and 0.089±0.060, respectively).

The amount of genetic variation in a population largely depends on the effective population size (Ne) and history of the population. Population size in Nepticulidae are known to vary greatly from generation to generation (Menken and Wiebosch-Steeman, 1988). Yet their populations contain large amounts of variation. This may be accomplished if much gene flow occurs thus expanding the population sizes. However, a closer look at the population structure hints at a highly differentiated pattern indicating low levels of gene flow.

Often conspecific populations of nepticulids are highly differentiated, showing large genetic distance values (up to 0.12, whilst usually intraspecific values amount to 0.05 or less; Thorpe, 1982; Menken and Ulenberg, 1987). Consequently, FST values, the among population variance in allele frequency, are rather high. Such values can be used as indirect measures of gene flow (Wright, 1943; Slatkin, 1985; Menken, 1989). For example, FST in *E. subbimaculella* is 0.285, which results in an average level of gene flow of 0.63 individual per generation (Menken, 1989), too little to prevent neutral alleles at a locus from differential fixation (Wright, 1931). Likewise, populations of the parthenogenetic species *E. argyropeza* can be composed of quite different combinations of clones even over short distances (Menken and Wiebosch-Steeman, 1988). Furthermore, large populations do not form panmictic units. For instance, a population of *E. rubivora* exhibited statistically significant deviations from Hardy-Weinberg equilibrium at all three variable loci that were examined (Menken and Bakema, unpublished). Homozygotes were generally in excess at the cost of heterozygotes (Wahlund effect; Wahlund, 1928) which might indicate that non-interbreeding populations were sampled and treated as an interbreeding one.

Such a closed population structure of nepticulid species can be simply explained by their life cycles which promote highly localized population substructuring. First, females which pupate in the soil underneath the tree on which they have fed as a larva very likely deposit their eggs on that very tree. This is true for both parthenogenetic (Menken and Wiebosch-Steeman, 1988) and sexual (Menken, unpublished) species. Second, most oviposition is within 1-2 days after eclosion. And finally, direct observations indicate that these moths very rarely fly but instead run over leaves and hop from leaf to leaf. As isolated trees are regularly infested, however, (passive) long distance dispersal does occur.

Thus Nepticulidae bear characteristics which appear to facilitate rapid speciation (e.g., Futuyma, 1986); they occur in small isolated populations and have low levels of gene flow; they are ecologically specialized (monophagous) and this reduces interspecific competition; with few competitors there is much room for adaptive radiation; they feed on highly predictable host plants and this should not select for highly migratory species.

Various reasons may account for the near absence of recent speciation.

1) The extinction rate is high compared to the speciation rate. Although nepticulid species appear to be rather old this is not an a priori reason why this should be so.

The fact is that the probability of extinction of a taxon is independent of its prior duration because species fail to adapt to changing environments at a stochastically constant rate (Van Valen, 1973).

2) During the last glaciation only a few species survived which subsequently dispersed.

3) Closest related species were not investigated. This cannot be true for the well known Western European situation (Van Nieukerken, 1985).

4) Nepticulid species might have low vagilities but not really isolated populations. Although not very likely, population differentiation might be explained by differential selection and habitat choice. On the other hand, population isolation together with bottlenecks which occur regularly in Nepticulidae should lead to much lower heterozygosity levels (e.g., see Menken, 1987) unless effective population sizes are much larger because of gene flow. This discrepancy can be partly explained by the small number of loci on which heterozygosity estimates were based. Usually a negative correlation between the number of allozyme loci investigated and mean heterozygosity is found (Simon and Archie, 1985; Singh and Rhomberg, 1987).

In conclusion, species like *Ectoedemia* and *Stigmella*, that are ecologically specialized with low levels of gene flow and much population substructuring, are especially prone to speciation. Yet recent speciation has not taken place to a great extent except for species on *Quercus*, a group of trees that recently underwent speciation itself.

REFERENCES

Cronau, J. P. and S. B. J. Menken (1989): Biochemical systematics of the leaf mining moth family Nepticulidae (Lepidoptera). II. Allozyme variability in the *Stigmella ruficapitella* group. *Neth. J. Zool.* (in press).

Futuyma, D. J. (1986): *Evolutionary Biology*. Sinauer, Sunderland.

Menken, S. B. J. (1987): Is the extremely low heterozygosity level in *Yponomeuta rorellus* caused by bottlenecks? *Evolution 41*, 630-637.

Menken, S. B. J. (1989): Population structure dynamics and conservation of sexual and parthenogenetic butterfly and moth species. In: J. van der Made (ed), *Future of Butterflies*. Pudoc, Wageningen (in press).

Menken, S. B. J. and J. Brouwer (1984): Biochemical systematics and speciation in the leaf-mining genus *Ectoedemia* Busck (Nepticulidae, Lepidoptera). *Proc. XVII Int. Cong. Entomol.* 10.

Menken, S. B. J. and S. A. Ulenberg (1987): Biochemical characters in agricultural entomology. *Agric. Zool. Rev. 2*, 305-360.

Menken, S. B. J. and M. Wiebosch-Steeman (1988): Clonal diversity, population structure, and dispersal in the parthenogenetic moth *Ectoedemia argyropeza*. *Entomol. exp. appl. 49*, 141-152.

Nei, M. (1972): Genetic distance between populations. *Am. Nat. 106*, 283-292.

Nevo, E, A. Beiles and R. Ben-Shlomo (1984): The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. *Lec. Notes Biomath. 53*, 13-213.

Simon, C. and J. Archie (1985): An empirical demonstration of the liability of heterozygosity estimates. *Evolution 39*, 463-467.

Singh, R. S. and L. R. Rhombert (1987): A comprehensive study of genic variation in natural populations of *Drosophila melanogaster*. II. Estimates of heterozygosity and patterns of geographic distribution. *Genetics 117*, 255-271.

Slatkin, M. (1985): Gene flow in natural populations. *Ann. Rev. Ecol. Syst. 16*, 393-430.

Thorpe, J. P. (1982): The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. *Ann. Rev. Ecol. Syst. 13*, 139-168.

Van Driel, J. W. and S. B. J. Menken (1988): Biochemical systematics of the leaf-mining moth family Nepticulidae (Lepidoptera). I. Allozyme variability in the *Stigmella betulicola*, *S. lapponica* and *S. marginicolella* groups. *Entomol. scand. 19*, 131-142.

Van Nieukerken, E. J. (1985): A taxonomic revision of the Western Palearctic species of the subgenera *Zimmermannia* Hering and *Ectoedemia* Busck s. str. (Lepidoptera, Nepticulidae), with notes on their phylogeny. *Tijdschr. Entomol. 128*, 1-164.

Van Valen, L. (1973): A new evolutionary law. *Evol. Theory 1*, 1-30.

Whalund, S. (1928): Zusammensetzung von Populationen und Korrelationserscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. *Hereditas 11*, 65-106.

Wilkinson, C., G. Bryan, S. B. J. Menken and E. J. van Nieukerken (1983): A clarification of the status of four taxa in the *Ectoedemia angulifasciella* group (Nepticulidae: Lepidoptera). *Neth. J. Zool. 33*, 211-224.

Wright, S. (1931): Evolution in Mendelian populations. *Genetics 16*, 97-159.

Wright, S. (1943): Isolation by distance. *Genetics 28*, 114-138.

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THE EVOLUTIONARY ASPECTS OF DUAL INHERITANCE IN MODULAR AND UNITARY ORGANISMS

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ABSTRACT

The DNA sequence and its methylation or other molecular patterns constitute two inheritance systems: the latter, i.e. the epigenetic inheritance (EI) controls the patterns of gene activity transmission between generations of cell populations during ontogenesis. The errors of EI and the DNA x EI x Environment interactions can contribute to rapid reorganization of the genome, especially in plants (in general in modular organisms). These are able to reflect environmental effects in modular organisms, where there is no distinct germ-line/soma sequestration. The possible evolutionary effects of EI include mutations, alterations of gene interactions, epigenetic constraints, rate- and direction changes. It is proposed that rapid cell lineage changes in plants or in the germ-line of insects driven by the changes of dual inheritance can induce dynamic coexistence or its lack between insects and plants specifically, and between modular and unitary organisms in general.

Key words: environment x DNA methylation x DNA interactions, cell lineage evolution, ecological specialization

THE PROBLEM

The comparative developmental genetic aspects of interactions of modular and unitary organism in general, and the insect-plant relations specifically represent new challenges.

Some basic problems of the insect-plant interactions can be regarded as a subcase of the more fundamental organizational problems of the interactions of modular and unitary organisms, because number of similarities can be recognized between the organization of insect-unitary versus plant-modular pairs, respectively. The study of comparative aspects of such organisms can throw light on cardinal genetic aspects of plants and insects, on which more specific, derived properties are stratified. These include some important determinants of evolutionary diversity of insect and plant developmental pathways and their interactions.

Modular and unitary organisms differ in several respects. In contrast with the unitary organisms, modular organisms consist of sequentially repeated developmental units (modules), their germ-line/soma sequestration is lacking, ontogenetically tolerated somatic mutations can occur, propagating differentially within and between

cell lineages (Jackson et al., 1985). The differential spread of somatic variation is of evolutionary importance, which is controlled by a variety of mechanisms (Buss, 1987), generating dynamic coexistential regimes within and between cell lineages in one or more individuals. Some multicellular protists, fungi, plants and 19 animal phyla have clonally reproducing, modular architecture, with unpredictable morphology and without active movement. The insects are unitary organisms. For botanists, all clonal organisms are modular at some organizational level.

As regards the analysis of determinants of diversity in modular and unitary organisms and their interactions, two main classes of evolutionary analyses can be distinguished. The bottom-up approach aims at elucidating the developmental factors affecting modular and unitary organization. Buss (1988) has shown, for instance, that in 22 extant metazoan phyla, the number of species is proportional to the timing of germ-line determination. Ecologists try to derive the range of biological diversity in terms of ecological principles, using top-down approaches. It is necessary to use both research lines, being the developmental generation of variation and its fixation or differential spread unseparable processes, at least in modular organisms.

Since the collective developmental transformations in populations are key steps in evolution, firstly I outline the nature of their inheritance in terms of two interacting inheritance systems. Then the evolutionary consequences of the dual inheritance are discussed in modular and unitary, or plant and insect organisms. Finally, the possible relevance of the dual inheritance for the evolution of ecological specialization is considered.

DUAL INHERITANCE

The DNA sequence and its methylation or other molecular patterns constitute two inheritance systems (see Holliday, 1987, for a review); the latter is the epigenetic inheritance (EI), which mainly consists of methyl groups on the cytosin. EI is inherited in a clonal or semiconservative way.

Because much of the DNA is unprotected by methylation, the ancient hypothetical protective function of EI is uncertain. EI controls cellular inheritance during development, as a transcriptional regulator, maily repressor. Among other characteristics, EI is of cell-, tissue-, age- and species- specific nature (Vanyusin, 1984). Therefore, it seems plausible that EI plays a role in cell differentiation and/or its spatial organization, i.e. pattern formation, life history variation, and species-specific characters, including their environmental reactivity.

DUAL INHERITANCE OF DEVELOPMENTAL TRANSFORMATIONS IN MODULAR AND UNITARY ORGANISMS

The sites of the generation of variation are the soma and the germ-line and/or reproductive cells in plants, where there is no distinct germ-line. Buss (1983, 1987)

has put forward the idea that in modular and especially clonal organisms, i.e. in many multicellular protists, plant, fungi and 19 animal phyla, Weismann's doctrina (the sequestration of germ-line and soma) is invalid. Weismann's dogma is the basis of the modern genetics, on which the neo-Darwinian evolutionary theory rests. Buss has also argued that:

1. There can be reversible transitions between somatic and reproductive cells in modular organisms.

2. The ontogenetic timing of the germ-line/soma sequestration varies among species.

3. The ontogenetic timing of the germ-line/soma sequestration defines the extent to which genetic variation arising in the course of ontogenesis is heritable. Buss (1983, 1987) has proposed a theory, which claims that the competition of different 'selfish' cell lineages within ontogenesis determines the polymorphism of developmental pathways of different organisms in various species.

Buss' theory does not provide molecular mechanisms for the explanation of the origin of germ-line ⇔ soma transitions. Furthermore, his theory can be extended to explain evolutionary interactions among modular and unitary organisms, including the insect-plant relations. The logical basis of this extension is a hypothesis on the existence of different dynamic regimes of cooperation and competition of cell lineages generating transmissible heritable variation within and between individuals (Molnár, in prep.). In other words, the dynamic coexistence of cell lineages can occur not only at the levels of individual ontogeneses, but within and between species as well. Cell lineage variations are, in part, responsible for the specificity of ecological interactions.

Therefore, it is necessary to reveal the origin, maintenance and transitions of the sources and extent of the heritable variation, propagating along germ-lines or equivalent cell lineages producing gametes. A test of this idea is the experimental perturbation of the cell lineages and the subsequent analysis of their ecological consequences, which is lacking in the theories of development.

A set of observations shows that EI plays a role in the cell differentiation in plants and animals (cf. Vanyusin, 1984), and the germ-line can transmit evolutionary modifications of EI in the form of changes of methylation (Holliday, 1987). Van Valen (1988) seems to be the first, who realized the importance of the changes of methylation in the differentiation of soma and germ-line, moreover the possible role of the errors in the processing of dual genetic information in a nonsystematic way, but in the context of Buss' theory. The following, more systematic evaluation of the evolutionary role of EI and its interactions with the DNA sequence in a given environment may throw light on the nature of generation and interactions of developmental variation.

Mutations of EI can arise as a consequence of its errors in its maintenance methylase activity (copying). These are epimutations to distinguish them from classical mutations (Holliday, 1987). The interactions of DNA and EI may result from spontaneous and environmentally induced DNA reorganizations by gene duplication, translocation, inversion, amplification and deletion. The most convincingly do-

cumented case, however, is that the pattern of methylation can influence the transposition of movable genetic elements (Fedoroff, 1989). The above listed alternative possibilities cannot be excluded, because they are able to displace the position of methylation pattern, which in turn influences not only classical mutations, but also recombination and its consequences in an indirect way. The change of methylation of the cytosin also permits the deamination producing a thymine (Bird, 1980).

EI influences differential gene activity as a part of an ontogenetically changing genome, hence can act as a compositional genetic constraint on the molecular heterogeneity of unitary and modular organisms. EI may therefore alter the branchings of alternative pathways of cell differentiation in development and evolution. This cell-autonomous constraint is complemented by anisotropic distribution of cytoplasmatic factors during cell division and cell interactions, which together generate cellular diversity within and between organisms.

The gametes of plants are produced from stem cells. In the transition between generative and somatic cells we need to reveal not only the possible role of DNA modification by methylation, but also the local and global electromechanochemical intercellular factors of this evolutionarily important process. It is known that the development of stem cells is asymmetric. This means that they give rise both to nearly identical and different cells, showing species specific characters (Wolpert, 1988). The cell lineages, producing reproductive cells also show species-specific traits, e.g. branching patterns (Klekowski, 1988). The generators of the asymmetrical stem cells and of the cell lineages arising from them, are of paramount importance in their selection acting at different levels of organization. In brief, my proposal is that EI may play an important role in the generation of asymmetries mentioned. If so, then the mutations of EI can cause rapid developmental changes, even during one generation.

The rate of evolution might also be influenced by EI. EI can increase the response to selection by inducing somatic variation. The variable timing and rate (heterochrony) of germ-line/soma sequestration is proportional to the number of possible classical and epimutations (mutations in the EI). Mutation rates and their constraints (e.g. repair) against mutational variation determine the rate of molecular evolution. Miyata et al. (1987) have shown that there is a positive correlation between the number of cell division before meiosis and the rate of mutation in sex-linked loci in mammals, which have more division in the male, than in the female. This fact reflects the differential contribution of male and female germ-line to the generation of DNA variations, which can also be observed in the case of EI, carrying asymmetrical methylation patterns in the two sexes (Surani et al., 1988).

There is a directionality in the transitions of somatic cells S_1, S_2,..., S_m to reproductive cells G_1, G_2,..., G_n in modular organisms, because the dedifferentiation of somatic cell types to germ cells can preserve some specific elements of the copying errors of methylation patterns of differentiated somatic cells. Considering the huge number of conversions between somatic and germ cells in the clonal organisms (cf. Buss, 1983, 1987), EI may play a substantial directional role in evolution, affecting the frequency of such cellular transitions.

Although the cell- and tissue-specificity of the patterns of methylation is normally erased during gametogenesis, the erasure of this 'genomic imprinting' does not work in a perfect way. Heritable errors of the erasure of methylation can be observed in natural situations (cf. Holliday, 1987). This can also be seen from the insertion of artificially methylated transgenes (Surani et al., 1988). The genomic imprinting is different in the patterns of methylation between maternal and paternal genomes, through which the parental genomes can influence the gene expression during the development of offsprings (see Solter, 1988, for a review).

If so, then any factor, including environmental effects, being able to modify the state of EI responsible mainly for genomic imprinting, can in principle be transmittable to offsprings, provided the induced changes will not be erased at the induced sites of question, arising from the errors of erasure. The existence of such a mechanism cannot be excluded in those modular organisms, where there is no germline/soma sequestration, which represents an ontogenetic barrier against the transmission of somatically stored environmental effects.

The response of the plant genome to environmental stress (e.g. heat shock) can be accompanied by the reorganization of the DNA sequence (McClintock, 1984; Walbot and Cullis, 1985) or of the chromatin (Cullis, 1987). Beyond the erroneous function of the erasure of methylation characterizing many dedifferentiated cells, which cannot be repaired in a perfectly reliable way, the genetic assimilation of the changes of methylation into DNA sequence, as can be demonstrated by Bird's experiments mentioned above, can reveal a new possibility for the evolution of genetic systems through DNA x DNA modification x Environment interactions. Dual genetic systems may operate via environment-dependent hormonal signals, e.g. via cytokinins (cf. Vanyusin, 1984).

EI may also illuminate some further phenomena:

1. if the association seems to lack between genetic and morphological changes;
2. if EI-induced interactions of loci and the subsequent advantages or disadvantages of heterozygotes or homozygotes exist in intra- or interpopulational interactions;
3. if connections exist between developmental genetic processes and population differentiation and/or speciation without apparent conventional 'genetic' changes.

These hypotheses imply that the effects of EI may have rather hidden forms sometimes, probably because of the almost unseparable Environment x EI x DNA interactions.

DUAL INHERITANCE AND THE EVOLUTION OF ECOLOGICAL SPECIALIZATION

The evolution of ecological specialization is the evolution of the fundamental niche, which is a 'manifestation of genetic properties of species' (Futuyma and Moreno, 1988, p. 208).

Since EI is also a part of the genetic systems, it is necessary to consider its possible ecological consequences.

One of the most interesting, unexplored experimental possibilities concerning the ecological reactions of the dual inheritance is the manipulation of EI and the subsequent record of niche width, norm of reaction, phenotypic or developmental plasticity to environmental effects in plants. Rapid reorganization in plant genomes and in their developmental dynamics is a well-known phenomenon (Walbot and Cullis, 1985), distinguishing specific plant characteristics, as opposed to the behavioral flexibility of insects (see also Sutherland and Watkinson, 1986, for a discussion of differences between plant and animal evolutionary strategies).

It is reasonable to expect that alternative developmental pathways map the differences of the dual inheritance onto niche differences in different ways. If so, then the important fact, that modular or clonal organisms sample their environments, can in part be connected to EI- variations. Because of EI-variations of the modules and their populations the plants are genetically of more mosaic nature (cf. Klekowski, 1988), than it was previously considered. This may cause or affect:

1. fine-grained niche subdivision even within clones,
2. a broader plant chemical spectrum to react to insects.

A consequence of the flexible dual genetic system is that the composition of the plants is a more dynamic input for the insects, than it was previously believed. Also, a preliminary investigation of models of coupled insect-plant life cycles shows, that the relative evolutionary rates of plant and insect cell lineages producing reproductive cells are important determinants of the insect plant dynamic coexistence.

These indicate a change of emphasis in the judgement of the insect-plant interactions. I stress the equivalent importance of the rapid changes of distribution of plant chemicals in the origin, maintenance and transitions of the host plant range, instead of previous scenarios enshrined, in which the flexibility of the insect recognition behaviour plays the dominating role. Rapid reorganization of the dual genetic systems in plants may be thought of as a cardinal determinant of the dynamic host range, when the mutation rate is large for secondary plant substances.

CONCLUSION

The aim of the propositions outlined in this paper is to initiate a developmental genetic approach to coevolution in general, and to insect-plant interactions specifically. The basic assumption is that many important aspects of coevolution are controlled by developmental changes within and between populations. A large number of developmental changes work via common mechanisms. The existence of the dynamic dual inheritance and of the DNA modification by methylation (EI) are such elements.

The EI is a further symmetry breaker of the asymmetrical elements of the genetic systems, which are amplified, suppressed or remain unaltered in certain environments. The cellular and cell lineage selection generating gametes operates as ampli-

fier and suppressor of the dual genetic variation contributing differentially to the spread of the individuals. The differential contribution of the cell lineages within and between modular and unitary organisms, specifically in plants and insects, can produce differential reproductive success as a result of their success in their internal and external environments. The differential spread of organisms having intrinsic generative potential is further amplified or suppressed or remains neutral by ecological pattern generation, creating an inhomogeneous biological world. I do not see how the paradoxical couplings of the divergent dynamics of plants and insects or of other coevolving creatures could come about. If we accept Jermy's view (this volume) on the stochastic nature of the recent insect-plant associations, then it is not implausible to consider the possibility of the coevolutionary relevance of the concepts of diverging nonlinear dynamic couplings (e.g. bifurcations, pseudorandom motions, etc.). Experiments can suggest nontrivial relations between the nonlinearities of olfactory EEG and the dynamics of plant chemical profiles affected by dual inheritance.

REFERENCES

Bird, A. P. (1980): DNA methylation and the frequency of CpG in animal DNA. *Nucleic Acids Res. 8,* 1499-1504.

Buss, L. W. (1983): Evolution, development and the units of selection. *Proc. Nat. Acad. Sci. U.S.A. 80,* 1387-1391.

Buss, L. W. (1987): *The Evolution of Individuality.* Princeton University Press, Princeton.

Buss, L. W. (1988): Diversification and germ-line determination. *Paleobiology 14,* 313-321.

Cullis, C. A. (1987): The generation of somatic and heritable variation in response to stress. *Am. Nat. 130,* 526-573.

Fedoroff, N. V. (1989): About maize transposable elements and development. *Cell. 56,* 181-191.

Futuyma, D. J. and G. Moreno (1988): The evolution of ecological specialization. *Ann. Rev. Ecol. Syst. 19,* 209-233.

Holliday, R. (1987): The inheritance of epigenetic defects. *Science 238,* 163-170.

Jackson, J. B. L., L. W. Buss and R. E. Cook (eds) (1985): *Population Biology and Evolution in Clonal Organisms.* Yale University Press, New Haven.

Klekowski, E. J., Jr. (1988): *Mutation, Developmental Selection and Plant Evolution.* Columbia University Press, New York.

McClintock, B. (1984): The significance of responses of the genome to challange. *Science 226,* 792-801.

Miyata, T., H. Hayashida, K. Kuma, K. Mitsuyasu and T. Yasunaga (1987): Male-driven molecular evolution: a model and nucleotid sequence analysis. *Cold Spring Harb. Symp. Quant. Biol. 51,* 836-867.

Solter, D. (1988): Differential imprinting and expression of maternal and paternal genomes. *Ann. Rev. Genet. 22,* 127-146.

Surani, M. A., W. Reik and N. D. Allen (1988): Transgenes as molecular probes for genomic imprinting. *Trends in Genetics 4,* 59-62.

Sutherland, W. J. and A. R. Watkinson (1986): Do plants evolve differently? *Nature 320,* 305.

Van Valen, L. M. (1988): Is somatic selection an evolutionary force? *Evol. Theory 8,* 163-167.

Vanyusin, B. F. (1984): Replicative methylation in animals and plants. *Curr. Top. Microbiol. Immunol. 108,* 99-114.

Walbot, V. and C. A. Cullis (1985): Rapid genomic changes in plants. *Ann. Rev. Plant Physiol. 36,* 367-396.

Wolpert, L. (1988): Stem cells: a problem in asymmetry. *J. Cell. Sci. (Suppl.) 10,* 1-9.

APPLICATION

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PLANT-LEAF MORPHOLOGY, HOST-PLANT RESISTANCE AND BIOLOGICAL CONTROL

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ABSTRACT

Host plant resistance and biological control are considered to be the corner stones of integrated pest management programs. To date, combination of these tactics by making a plant less suitable for the herbivore, and, at the same time, making it more attractive for natural enemies of the herbivore, has seldom been pursued. The study of tritrophic systems has largely been neglected until now, whereas such studies are essential in finding out whether the development of host-plant resistance might have negative effects on natural enemies. Breeding for insect resistance does change host-plant quality, which affects herbivore and natural enemy. Tritrophic interaction studies are also elemental in discovering how plants support the action of natural enemies and how the plant might be manipulated to the benefit of natural enemies. In this paper examples will be discussed to illustrate how plant morphology influences the pest reduction capacity of natural enemies.

Key words: plant leaf morphology, host plant resistance, plant breeding, biological control, natural enemy, parasite, tritrophic system, leaf hairs, leaf boundary layer, host plant selection, host plant preference, host plant quality, walking speed, walking pattern, searching efficiency, encounter probability, *Trialeurodes vaporariorum*, *Encarsia formosa*, cucumber, gerbera.

INTRODUCTION

Several books and articles dealing with aspects of tritrophic systems have been published recently (e.g. Boethel and Eikenbary, 1986; Dicke 1988; Juniper and Southwood, 1986; Price, 1986). The general trend of reasoning in this literature is that the study of tritrophic systems has largely been neglected until now. Such studies are essential, though, in finding out what role the plant plays in "supporting" the action of natural enemies, either or not in combination with host-plant resistance, and how this role might be manipulated to the benefit of natural enemies. It is generally accepted that insect resistance in plants is compatible with biological control, although most of the research in these two fields has been done independently (Boethel

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and Eikenbary, 1986). Bergman and Tingey (1979) reviewed the literature and found that the combined effectiveness of resistant hybrids and biological control had been studied in a few instances only and that their interaction remained poorly understood. Breeding for insect resistance changes plant quality, which in turn affects herbivorous as well as entomophagous species. Only through an understanding of the biological processes between the different trophic levels may we manipulate host-plant characteristics to maximize the combined control resulting from plant resistance and biological control. Plant breeders have emphasized mainly the study of, often unknown, intrinsic defense mechanisms (sensu Price, 1986), i.e. defense produced by the plant alone through production of chemicals such as toxins or digestibility reducers, or through physical defense by trichomes or toughness, or by a combination of the two, as with glandular trichomes or resins. Those studying biological control have emphasized the need for extrinsic defense mechanisms of plants (sensu Price, 1986), i.e. situations where the plant benefits from the natural enemies of herbivores. It is now recognized that mechanisms of intrinsic defense of a plant directly or indirectly can influence other trophic levels in a positive or negative way (Bergman and Tingey, 1979; Price, 1981). The details of direct and indirect effects of plants on herbivores and their natural enemies at the chemical level have received considerable attention in the last decades (e.g. Bell and Cardé, 1984; Nordlund et al., 1981; Shorey and McKelvey, 1977). The development of theory on three trophic level interactions, however, deserves more study (see e.g. Sabelis and de Jong, 1989). The application of knowledge from three trophic system studies to agricultural, forest and urban ecosystems is still in its infancy.

In order to develop theory and to apply knowledge, an integration of the different kinds of factors that mediate interactions, e.g. physical and chemical factors (Price, 1986), is needed. Awareness of plant effects higher up in the trophic system is essential in integrating plant breeding and biological control using natural enemies. To date, the often delicate and complicated aspects of tritrophic relationships are only touched upon (Price, 1986).

In this article we will concentrate mainly on how plant-leaf architecture influences the pest reduction capacity of natural enemies. This is illustrated with examples of the relationship between plant-leaf hairiness and searching efficiency of parasitic wasps. Before presenting results from our own research, we will summarize the literature on plant hairiness related to natural enemies.

LEAF CHARACTERISTICS, HOST-PLANT RESISTANCE AND BIOLOGICAL CONTROL

Leaf characteristics. First, let us look at the plant-leaf surface's functions. In the foreword of their book, Juniper and Southwood (1986) write: "For the plant, its surface is not merely the point of first contact with insects, but also its barrier to the external world as a whole. It is a barrier, but it is a permeable, translucent barrier through which both energy and the essential nutrients of a plant, gaseous and liquid,

enter and from which emerge, amongst others, signals, inducements and repellent substances. These vulnerable areas are then defended by numerous surface mechanisms. Thus a plant's surface will be fashioned in evolution not only by the interplay of the physical environment with the physiological needs of the plant, but also by the dictates of defence and the opportunities for mutualistic interactions." In the first chapter of the same book some reference is made to the interaction between plant and natural enemies, in that Southwood (1986) mentions that natural enemies are influenced by hairs (size, density and alignment) and that the degree of effect is related to the size of the insect and its mode of locomotion. Jeffree (1986) adds that: "The plant surface ... is evidently a functional organ, and presumably the diversity of plant surface structure is a reflection of the diversity of environmental pressures by plants." Cuticles are not simple homogeneous structures, like films of varnish, but have a layered structure, representing regions of non-uniform physical properties and chemical composition, and cuticle structures show great variability. A layer of epicuticular wax generally coats the cuticle proper. Wax layers vary in thickness and in structure. A structure analysis with TEM and SEM has only been possible for two decades. Many types of structures were found, varying from amorphous films to substantial crusts. Functions of wax are supposedly waterrepellency and waterproofing. In the frame work of tritrophic relationships it is interesing to know that waxy plant surfaces are able to adsorp and re-release insect sex pheromones (Colwell et al., 1978; Wall and Perry, 1983). Such adsorption may result in the attraction of males. Further it was shown recently that airborne sex pheromone, released by individual calling *Mamestra brassicae* moths, is absorbed to cabbage leaves. The absorbed pheromone affects the behaviour of conspecific male moths as well as that of a natural enemy, the parasite *Trichogramma evanescens* (Noldus, 1989).

There is little clear evidence of the functional differentiation of epicuticular waxes relevant to plant-insect interactions, but this area is not well enough studied (Jeffree, 1986).

Plant hairiness. More information is available on the relationship between plant hairs and insects. The range of structural types of trichomes is immense (Jeffree, 1986). They can be single-celled or multicellular, and both types can be glandular or non-glandular, etc. The functions of hairs are very diverse. They can be used for (1) climbing on other plants, (2) shedding water (3) preventing desiccation and reducing transpiration, (4) providing optical qualities, and (5) for hampering and trapping insects.

Various hair types influence insects differently: stiff downwards pointing hairs form a mechanical protection against crawling insects or molluscs, felted trichome layers form physical barriers. Hooked trichomes entangle insects, and glandular trichomes trap insects through adhesive excretion and kill them with toxic fluids.

Southwood (1986) mentions the following effects of hairs on insects:

(a) *effects at a distance*: odour, reflectance (i.e. colour to the insect) and shape are three factors that influence behaviour of insects in the proximity of the plant.

(b) *effects when on the surface*:

direct effects: hairs may form a hurdle to attack the surface, the leaf surface

may be too smooth, or too hairy for attachment. Hairs have very different influences, sometimes they create problems, at other times they provide foothold. Hairs may frustrate insect movement through simple impedance, through physical trapping by hooked hairs, or through sticking through the exudates from glandular trichomes. The type of reaction depends on hair properties as length, density and alignment (erect, procumbent, etc.), and on features of the insects such as size, mode of locomotion and mode of feeding. The effect of a certain hair density and hair type is also a matter of size of the insect. Further, hairs may be repulsive or toxic, or they may attract and/or retain insects.

indirect effects: indirect effects can be manyfold, e.g. the target insect can be influenced by the microclimate created by specific leaf characteristics or by other organisms. The other (e.g. non-phytophagous) organisms are often differentially influenced by characteristics of the plant hairs because of other size ratios. The microclimate - which can have different effects on herbivores and natural enemies - is strongly affected by hair type and density.

The influence of the microclimate in the leaf boundary layer is treated in detail by Willmer (1986) who formulates that: "... plant surfaces thus develop a boundary layer of relatively still air where winds have little effect and where temperature and relative humidity effects can be markedly different from ambient. For insects these gradients, around any surfaces, are especially relevant because the boundary layer may be only millimetres or at best a few centimeters deep, so spanning precisely the zones in which resting insects will be found. Transpiration normally raises the humidity, and may lower temperatures too, especially around lower leaf surfaces; thus gradients can be more favourable to a resting or feeding insect." Both, herbivores and natural enemies, may profit from the conditions in the boundary layer. The boundary layer and the upper surface of the leaf can be up to 7°C warmer than the ambient air when exposed to sunlight. During the day the under surface is usually a few degrees cooler than the air temperature. At night the temperature of the under surface is about equal to ambient temperatures, while that of the upper surface may be lower. Due to reflectance, the under surface may be heated by radiation from the soil, and the temperature thus be somewhat higher than ambient. The under surface, especially, provides a cool and moist niche of reasonable constancy throughout the 24-hr cycle (Willmer, 1986). The dependence to living in the leaf boundary layer can be such that "... larvae that live in favourable microhabitats normally have their waterbalance controlled by that habitat, and have to invest much less in other forms of protection such as impermeable cuticle and complex osmoregulatory response" (Willmer, 1986).

Leaf morphology and biological control: examples. An evaluation of the scarce liturature on host-plant resistance based on leaf hairiness in combination with biological control teaches us that the relationship is not always simply positive and leading to better pest control. For example, an increase in the number of glandular hairs makes potato plants more resistant to aphids, but at the same time it has an adverse effect on the natural enemies of aphids (Obrycki, 1986). The natural enemies

get covered also or are entrapped by the trichome exudate. Obrycki (1986) therefore proposes to breed hybrids with moderate densities of glandular trichomes for use in the development of an IPM program for potatoes. In cotton plants, which are attacked by several species of pest insects, an increase in hairiness may reduce numbers of one species but increase that of other species (Mound, 1965; Pollard and Saunders, 1956). There is a number of other examples in the literature which illustrate that natural enemies are hampered by dense hair layers. E.g. Schuster and Calderon (1986) show an inverse relationship between cotton trichome density and parasitization of *Heliothis* by *Trichogramma* and predation by *Chrysopa*. Breeders of plants resistant to herbivores should, therefore, also examine the effect of changes in host-plant characteristics on natural enemies in order to (1) identify potential adverse interactions and to (2) enhance positive interactions. To which Obrycki (1986) adds: "It remains for an enlighted plant breeding research group to develop lines which not only defend against pests but also promote natural enemies", and: "The benefits of using these two tactics in compatible and complementary ways to promote both the stability of resistant varieties and effective long-term pest management far outweigh the research costs required during the development of these integrated programs."

Partial resistance can, however, contribute considerably to biological control. Kaneda (1986), for example, studied the relationship between rice, brown planthopper (*Nilaparvata lugens*) and its natural enemies. He concludes that partial resistance alone leads to some pest reduction, but in combination with the naturally occurring predators (mainly spiders) it leads to effective suppression of brown planthopper under the economic threshold level. Van Emden (1986) gives other examples showing the same effect as illustrated by Kaneda. He further gives an explanation for the finding that partial insect resistance often shows more clearly in field than in greenhouse tests: in the field natural enemies add to the reduction of herbivores, and it is thus not only partial resistance which is measured in the field. But van Emden (1986) also describes a set of experiments in which opposite results were found: natural enemies were observed more often and had a greater effect on the susceptible lines than on the resistant lines: it was suggested that the resistant lines produced lower concentrations of attractive allelochemicals for the natural enemies than the susceptible lines. This, again, warns us that breeding for resistance does not automatically lead to better overall pest control, but, contrarily, may result in a reduction of attractiveness for natural enemies. Work on tritrophic systems which was only embarked upon a short time ago, is expected to generate a wealth of information on the physical and chemical relationships between the components of the systems (see e.g. Barbosa and Letourneau, 1988; Dicke, 1988; Noldus, 1989).

ENCARSIA FORMOSA, WHITEFLIES AND DIFFERENT HOST PLANTS

Let us now turn to a case study: the influence of leaf hairiness on the parasitic wasp *E. formosa*.

The whitefly problem. Whiteflies (Homoptera: Aleyrodidae) rank among the most noxious insects attacking field and greenhouse crops around the world (Mound and Halsey, 1978). Larval instars as well as adults feed on the phloem sap of hundreds of species of plants and cause damage by the excretion of honeydew that falls on leaves and fruits and serves as a suitable growth medium for fungi, that hamper photosynthesis and render fruits unmarketable. Furthermore, whiteflies can act as vectors for virus diseases. Whiteflies are the aphids of the tropics (Mound and Halsey, 1978). Pesticide resistance makes chemical control of whiteflies increasingly difficult. The development or improvement of non-chemical control methods, however, requires a thorough understanding of the interactions between the insects and their host plants as well as the relations between whiteflies and their natural enemies. It is only recently that collection of such information started, but the rewards have been very satisfactory so far. Host-plant resistance and biological control form the cornerstones of integrated pest management (IPM), which is examplified by the IPM programs applied in greenhouses (van Lenteren and Woets, 1988). However, the greenhouse whitefly cannot be controlled effectively on all important crops (see below) and we expect fundamental research to provide us with ideas to improve biological control, e.g. through host-plant resistance enhancing the reduction effect of the natural enemy, or to change host-plant characteristics augmenting parasite searching efficiency.

Understanding host-plant selection and population dynamics of whiteflies and natural enemies requires basic behavioural and ecological information (van Lenteren and Noldus, 1989; Noldus and van Lenteren, 1989). The relationship between selection of oviposition sites and growth, survival and reproduction of offspring is a central element in the evolution of host associations between herbivorous insects and plants (Singer, 1986; Thompson, 1988). Oviposition site selection by female whiteflies has a profound effect on their fitness, as larval stages are largely sessile. As most whiteflies feed and oviposit on the same leaves, feeding and oviposition site selection go hand in hand. Thus, whiteflies should select those plants and plant parts that are most suitable for feeding *and* oviposition.

When searching for feeding and oviposition sites in flight, the two whitefly species which are key pests in greenhouses, the greenhouse whitefly *Trialeurodes vaporariorum* and the cotton whitefly *Bemisia tabaci*, react only to a limited range of colours. For both whitefly species no long-distance responses to any other plant characteristic than colour have been found. Attraction to ultraviolet light has been recorded for *B. tabaci* and is supposed to indicate a dispersal phase. Attracted by yellow-green, whiteflies alight on potential host plants, but actual selection of a host plant, or part of a plant, occurs after landing. Whiteflies make short probes in the leaf tissue just below the epidermis (van der Kamp and van Lenteren 1981; Noldus et al., 1986). Contact with phloem vessels - the primary feeding source - is not necessary for the selection of a suitable host plant or feeding site within a plant (Noldus et al., 1986; Janssen et al., 1989). The host plant-preference pattern of the greenhouse whitefly correlates well with host-plant suitability: whitefly population growth is much faster on plants preferred for feeding and oviposition than on unpreferred plants. Within

a host plant, whiteflies prefer young leaves (Noldus et al., 1986; Ohnesorge et al., 1980), and they arrive at such leaves after a process of between-leaf movement and probing. Naive females stay longer on young leaves than on old ones. On old leaves they move and probe more frequently, spend less time probing and feeding, and depart sooner (this behaviour is also observed on less preferred host plants, van Lenteren et al., 1977). Residence times on old leaves are further decreased after prior experience on young leaves. Female *T. vaporariorum* transferred from old to young leaves spend significantly more time probing and feeding and stay longer on young leaves. Prior experience thus clearly affects selection behaviour, which is a well-established phenomenon in phytophagous insects (e.g. Papaj and Prokopy, 1989).

Host-plant preference may evolve rapidly. There is evidence for both *T. vaporariorum* and *B. tabaci* that local populations may adapt to host plants - on which they initially developed poorly - within 3-5 generations (Dorsman and van de Vrie, 1987; van Lenteren and Noldus, 1989; van Lenteren et al., 1989a). Dorsman and van de Vrie (1987) found that whiteflies reared for a number of generations on gerbera develop faster, have far lower mortality and greater longevity on gerbera than whiteflies originating from the stock rearing on tomato and reared for the first generation on gerbera. This and other examples, also for *B. tabaci* (van Boxtel et al., 1978; van Lenteren and Noldus, 1989; van Lenteren et al., 1989a) indicates local selection for adaptation to relatively poor host plants.

We are beginning to understand how spatial distribution patterns of whiteflies in greenhouses arise. Movement of *T. vaporariorum* occurs mainly during the first few days after emergence over distances of a few meters only and the resulting distributions are highly aggregated. There is evidence for a dispersal phase in the early life of both *T. vaporariorum* and *B. tabaci* (van Lenteren and Noldus, 1989). Population growth of whiteflies is exponential as long as host plants remain in good physiological condition. The population growth rate of *T. vaporariorum* on a range of host plants can now be estimated on a number of host plants, by means of simulation models based on autecological data (Hulspas-Jordaan and van Lenteren, 1989, Yano et al., 1989a,b). Data on host-plant suitability - in terms of whitefly population growth rate - are definitely needed to judge the result of plant breeding efforts in the field of host-plant resistance and to estimate the success of biological control. For an extensive review of selection by and suitability of host plants for whiteflies, see van Lenteren and Noldus (1989).

As mentioned before, not all pest and disease problems in greenhouses can yet be controlled by either host-plant resistance or biological control. In cucumber crops, for example, one of the key insect pests, the greenhouse whitefly *T. vaporariorum*, is difficult to control through seasonal inoculative releases with the parasite *E. formosa*, whereas control on tomato is easily obtained. Whiteflies can be controlled successfully on cucumber on the premise that parasites are released weekly over a long period, but this is economically not attractive.

Table 1 Leaf hairiness (data from van Lenteren unpublished) and the walking speed of *Encarsia formosa* (data from Hulspas-Jordaan (1977), except for half hairy cucumber which are from Li et al. (1987)).

Plant species/plant type	No. of hairs per square cm average ±s.d	Type of hairs	Walking speed in mm/sec ±s.e.
Cucumber haired	382±147	straight, higher than wasp	0.21±0.02
Gherkin	213±36	straight, higher than wasp	0.28±0.03
Tomato	1943±284	curved, much shorter than wasp	0.30±0.03
Gerbera	107±19	curved, longer than wasp	0.34±0.03
Egg plant	198±28	umbrella shaped, higher than wasp	0.37±0.05
Cucumber half-haired	156±64	straight, higher than wasp	0.40±0.01
Cucumber hairless	0		0.63±0.09
Sweet pepper	0		0.80±0.07

Leaf hairiness and biological control of whitefly on cucumber.

Introduction. We presume whitefly control on cucumber to fail because of two reasons. First, cucumber is a very good host plant for greenhouse whitefly (van Boxtel et al., 1979; van Lenteren and Noldus, 1989). Secondly, *E. formosa* has difficulties in finding whitefly larvae on the densily haired cucumber leaves. The effect of leaf hairs on walking speed and walking pattern of *E. formosa* was first demonstrated by Hulspas-Jordaan and van Lenteren (1978): the walking speed on cucumber hybrids is inversely proportional to the hairiness of the leaves (Table 1).

Further, the parasites turn more on hairy leaves, which often leads to searching on previously visited parts of the leaf. Finally, because honeydew excreted by whitefly larvae is retained much more on hairy than on smooth leaves, the parasites run more often into droplets of honeydew, and then either spend hours preening or drown and die in the honeydew. Time used for preening can, of course, not be used for

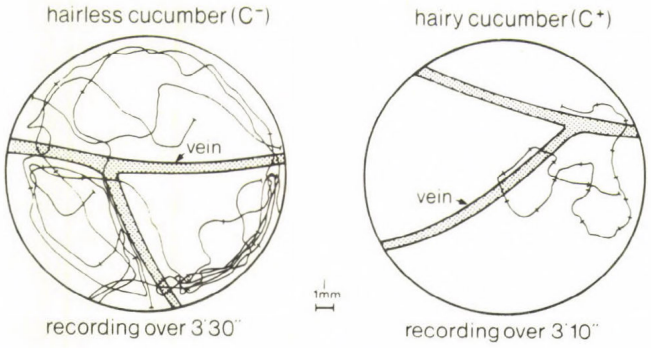

Fig. 1 Walking pattern of *Encarsia formosa* on hairy (C+) and hairless (C-) cucumber leaves. The circle is the inner outline of the perspex ring, the veins of the leaves are dotted. The cross-lines on the walking pattern indicate the position of the wasp every ten seconds (after Hulspas-Jordaan and van Lenteren, 1978).

searching hosts. As *E. formosa* females search randomly for hosts once landed on a leaf, the encounter probability can be easily estimated and is the result of walking speed and pattern, and the circumference of a larva relative to that of other larvae on the same leaf (van Lenteren et al., 1976, 1980).

O. M. B. de Ponti (unpublished) examined circa 200 cucumber accessions on the degree of hairiness and hair length. Although he found variation in the two characteristics, this work was terminated because he traced two hairless mutants in the literature (*Cucumis sativus* L. cv. Odnostobelnyi and cv. Mayak, the latter was named cv. "IVT No. 761077 Mayak" afterwards, de Ponti, 1984). With these mutants we were able to show that walking speed dramatically increased (3 times faster than on hairy cucumber), that parasites walked more straight than on hairy leaves (Hulspas-Jordaan and van Lenteren, 1978), and that the net area searched per unit of time was 3.5 times larger on the hairless cucumber (Fig. 1, Hulspas-Jordaan, 1977). The problem on these completely smooth leaves was, however, that *E. formosa* walked so fast that they walked over whitefly larvae without noticing them. Thus, a breeding program was set up to select for cucumber lines with a lower number of hairs than the commercial hybrids used at that time. It appeared rather simple to develop hybrids with half the number of hairs, hair size and -shape remaining about equal (Inggamer and de Ponti, 1983) as hairiness is governed by one dominant gene with intermediary inheritance. This is in agreement with Southwood's (1986) remark that: "where it has been investigated, the extent of pilosity is under relative simple genetic control, one or two genes." We compared the searching efficiency on these "half-haired" hybrids with that on commercial normally haired hybrids in a laboratory set-up where parasites had to walk at least 2.5 cm on the leaf surface before being able to contact a whitefly larva (Fig. 2, Li et al., 1987). In this paper we will use the terms "haired" and "half-haired" to indicate commercial hybrids with the normal

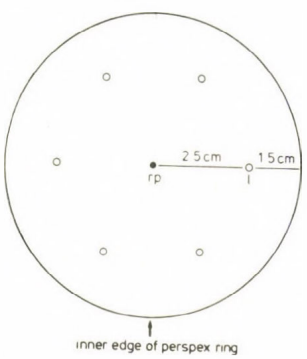

Fig. 2 Distribution pattern of six whitefly larvae on a leaf part enclosed by a perspex ring; rp = release point of *Encarsia formosa* (after Li et al., 1987).

Table 2 Host-finding success and host-searching activity of *Encarsia formosa* on two cucumber hybrids in an arena on the leaf (after Li et al., 1987).

Cucumber hybrid	% of wasps that found a host larva	Searching time till finding a host larva (in seconds)
Cucumber haired	56	1564
Cucumber half-haired	77	1111

number of hairs, and the new hybrids with a lower number of hairs respectively; we realize this to be a qualitative description.

Parasites needed significantly less searching time to find larvae on the half-haired hybrid than on the hairy ones (Table 2). The walking speed was significantly higher on the half-haired hybrid, the walking patterns did not differ significantly on both leaf types.

An important aspect to know is whether the hairless, half-haired and normally haired leaves have the same host-plant quality for the greenhouse whitefly. Host-plant acceptance, rate of development from egg to adult, mortality during development and fecundity were the same on the different cucumber leaves (O. M. B. de Ponti and J. C. van Lenteren, unpublished; van Rongen, 1979; van Venetie, 1978). The degree of hairiness does not apparently influence performance of *T. vaporariorum*. This might seem to be in contrast with results obtained with the cotton whitefly *B. tabaci*, where the flies develop much better on hairy than on smooth cotton hybrids in the dry and hot Sudan Gezira (Mound, 1965). However, the experiments on cotton were done in the field, at low ambient humidities, and the better performance of *B. tabaci* on hairy hybrids is explained by a more suitable microclimate for whiteflies. Relative humidity in greenhouses is always high and never a limiting factor for whiteflies. *Cucumis* species with a very high hair density make oviposition and feeding by *T. vaporariorum* impossible, but due to strong crossing barriers between *Cucumis* species it was difficult to transfer this characteristic to *Cucumis sativus* (Kowalewski and Robinson, 1977).

The observation that hairs are hampering small predators and parasites has been demonstrated on several crops (Elsey, 1974; Obrycki et al., 1983; Rabb and Bradley, 1968). On cucumber, larvae of the aphid predator *Coleomegilla maculata* are much hindered by the hairs and often fall off the leaf (Gurney and Hussey, 1970).

The last step in testing the hypothesis that hairs impede the walking speed and have an effect on the degree of parasitism should be an experiment at a much larger scale: in the greenhouse. Such experiments are, however, difficult to perform as we experienced in the past five years. In the following paragraphs we summarize the development to a final, successful experimental set-up. The experiments were all done with cucumber plants which differed in the number of hairs per unit of leaf area.

The main aim of the greenhouse test was to develop an experimental set-up in which the inluence of varying hair densities could be tested reliably and rather fast. The results of initial greenhouse tests were inconsistent due to the procedure we

followed for the tests. In this procedure the half-haired and haired cucumber hybrids were planted in two similar greenhouse compartments were they developed for several months. Then adult whiteflies were introduced in leaf cages for several days to lay eggs. Whitefly eggs were allowed to develop until the third larval stage (the preferred stage for oviposition by *E. formosa*, van Lenteren et al., 1980) which takes circa three weeks. Subsequently, parasites were introduced which were allowed to oviposit unlimitedly. Then the population dynamics of both whiteflies and parasites were followed during several generations. Not only were these experiments very time consuming, but also there were always problems with desynchronization of development in two different compartments during the relatively long experimental period of 5-6 months. Additionally other pests could immigrate making chemical control necessary, etc.

Because of all these problems, we decided to use a rather different set-up which is described below.

Final set-up of the parasitization experiments in greenhouses. The commercial cucumber hybrid Farbio, and the half-haired hybrids IVT no. 83262, 83264, 83265 were used. The half-haired hybrids had the same mother line and different but related father lines: thus genetic differences were small. Hair density was determined by counting the number of hairs at the leaf underside of leafdiscs. Per hybrid 21 leafdiscs were analyzed; 3 discs per leaf of seven different leaves of leaf layer 4 from the bottom, which was the leaf layer (leaf age) on which whitefly larvae were present during the experiment. Hair shape and size is the same on half-haired and commercial hybrids.

Plants were reared in a whitefly and *E. formosa* free greenhouse. Whitefly infestation was obtained by putting one whitefly female during 24 h in one leaf cage per plant, on the 4th leaf from below. The whiteflies originated from a stock rearing started in 1970 and kept since on the related tomato hybrids "Moneydor" or "Moneymaker" (van Lenteren and Noldus, 1989).

When the whitefly larvae were in a suitable stage for parasitization, the cucumber plants were transferred to two identical experimental greenhouse compartments of 6x18 m. In one compartment only plants of one type were placed. The position of the half-haired and haired plants was switched after each test. The plant distance within a row was 0.5 m and between rows circa 1.5 m. This is a somewhat lower plant density than that used under commercial conditions, but it enabled us to check the plants for presence of parasites without touching plants. Fifty plants, five rows with 10 plants, were put in the compartments.

During evening twilight a glass vial containing one *E. formosa* female of circa 24 h old was placed at soil level near the stem of each cucumber plant. The females, originating from the commercial rearing of Koppert BV, had not parasitized before but did have food (honey) available from the moment they had emerged. Vials were opened and wasps could walk out. Forty-four hours after release parasite adults were killed by a treatment with hydrocyanic gas. Dissection of the whitefly larvae took place three days later. Parasitized, unparasitized and host fed larvae were distinguished.

Table 3 Numbers of hairs on different cucumber hybrids (n=21 per line) (after Kamerman, 1987)

Cucumber hybrid	Number of hairs per cm^2 ± standard deviation
cv. Farbio	157.8±23.9
IVT No. 83262	74.8±17.6
IVT No. 83264	65.2±10.4
IVT No. 83265	59.6±12.7

Night temperature in the compartments was 18°C (dark period circa 7 pm - 7 am), day temperature was 22°C except for sunny periods when the temperature occasionally increased to maximally 24°C. The experiments were done in a 2-month period during autumn 1987.

Results of the parasitization experiments in greenhouses.

Hair numbers. The numbers of hairs found for each hybrid are given in Table 3. The numbers of hairs on the half-haired hybrids were approximately half of those of the commercial hairy hybrid, or even less. The hair numbers are about half of those presented in Table 1. The numbers are lower because hair density was determined on older, fully extended leaves.

Host killing and parasitization capacity of E. formosa. Encarsia formosa does hardly search and parasitize at night (Hoogcarspel and Jobsen, 1984). The total number of hours with daylight during which wasps could lay eggs was 12 on the first and 8 on the second day. During these 20 hours, and with the temperature regime used, an *Encarsia* female can lay 10-15 eggs (van Vianen and van Lenteren, 1986). The numbers of whitefly larvae present at the moment of parasitization varied between 168 and 385 per compartment among experiments and was about the same on half-haired and haired cucumber within an experiment (van Lenteren et al., 1989b). The intro-

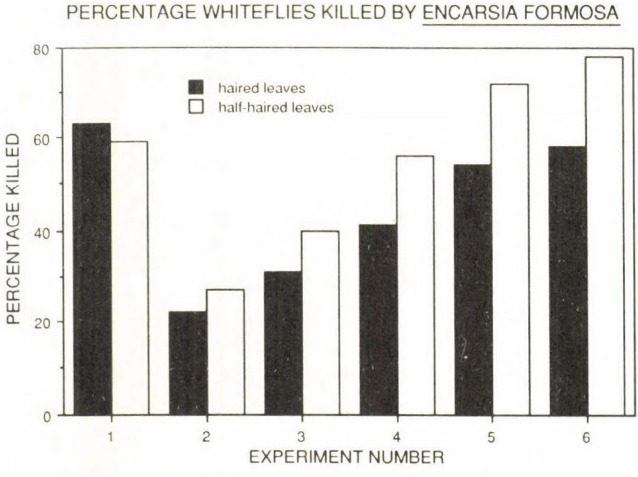

Fig. 3 Percentage of whitefly larvae killed by *Encarsia formosa* either through parasitism or through host feeding. Paired-samples t-test: reject zero-hypothesis of equal parasitism on both cucumber lines ($P<0.05$) (J. W. Kamerman, J. C van Lenteren and Z. H. Li, unpubl.).

duced *Encarsia* females had a total oviposition capacity of at least 5000 eggs (50 females x 10 eggs), thus a limited oviposition capacity cannot have caused a percentage of parasitism lower than 100%. In this experiment differences in percentage parasitism can only be the effect of a difference in searching efficiency of the parasites on leaves with a varying degree of hairiness.

In Fig. 3 the total host killing and parasitization capacity of *E. formosa* is given as percentage of the number of hosts present. The host killing and parasitization capacity of *E. formosa* is better on half-haired than on haired cucumber.

DISCUSSION

Plant characteristics and parasitization efficiency: The results presented in this article show how, through a breeding program to decrease the hairiness of leaves, the percentage parasitism of whiteflies by the parasite *E. formosa* can be increased significantly, thereby improving biological control of this pest on cucumber.

The experimental set-up we have developed, makes it possible to perform tests relatively fast and with little risk of complication (e.g. pesticide interference, infestation with whiteflies, other pests or natural enemies). Compared with the earlier experimental set-ups this final test was a great improvement with the benefit that testing still occurred under a semi-commercial production situation. The test remains time-consuming, but the outcome is of such importance that the investments are worth the effort.

The whole sequence of experiments (walking speed and pattern, time needed to discover a whitefly, whitefly encounter probability and the parasitization test in the greenhouse) leads to the conclusion that an increase in the number of hairs (of the size and type occurring on cucumber: unicellular, stellate and 0.40 mm long) leads to a decrease in encounter probability and encounter rate.

For cucumber we now have a quite complete understanding of the intricate relationships between parasite - host - and host plant. Based on this understanding we were able to direct breeding efforts and we now have breeding lines available for commercial breeding companies.

Leaf hairiness and biological control of whitefly on gerbera. It is only recently that attempts for biological control of insect pests of ornamentals have started. The main limitation is the almost zero tolerance for insects or signals of their previous presence like empty larval or pupal skins, empty mines or other signs of damage (van Lenteren and Woets, 1988). In such a situation biological control is difficult.

Increasing pesticide costs, pesticide resistance, extensive pesticide use (Table 4), residues on the traded plants, health risks related to their application, and limited number of pesticides allowed for use in greenhouses all lead to a serious reconsideration of biological control in ornamentals. One of the IOBC/WPRS working groups is now actively exploring IPM programs for ornamentals. Countries which mainly sell their produce at their own market have made progress with application of IPM (e.g. Denmark and the U.K.).

Table 4 Pesticide use on greenhouse crops in the Netherlands (Berends, 1988)

Crop	Kilogram of pesticides (active ingredient) per hectare per year
Chrysanthemums	220
Roses	87
Gladioli	40
Tulips	29
Irises	24
Gerbera	18
Cucumber, lettuce, sweet pepper, tomato	10-16

* pesticides for soil sterilization not included

In the Netherlands we are now studying the possibility to use *E. formosa* for the control of *T. vaporariorum* and *B. tabaci* on gerbera (*Gerbera jamesonii*). Growers in Poland and Hungary had already informed us that on some gerbera cultivars biological control of whitefly seemed possible, whereas on others whitefly seemed to develop better and/or *E. formosa* parasitized less efficiently. Our own observations led to the idea that also in gerbera the degree and type of hairiness might play a role. To study this, the same approach was followed as for cucumber. The quality of several gerbera cultivars for *T. vaporariorum* was determined by Dorsman and van de Vrie (1987) and R. Dorsman (unpublished). The developmental rate, mortality, fecundity and life-span of whitefly on four cultivars (cvs. Clementine, Espace, Ap-

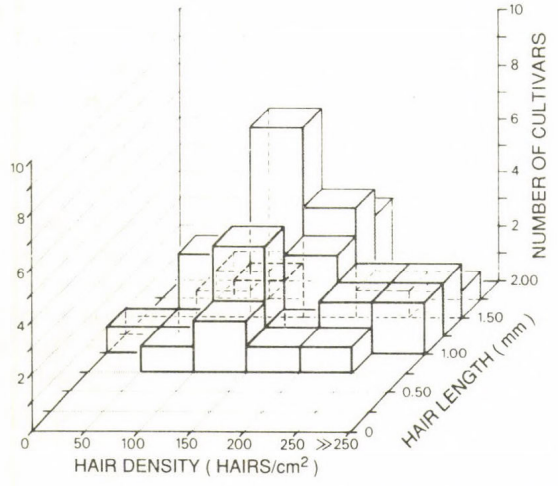

Fig. 4 Variation in hair density and hair length in *Gerbera jamesonii*. The vertical axis gives the number of cultivars with a certain hair density and hair length (after Godthelp, 1989).

Table 5 Developmental data of *Trialeurodes vaporariorum* on different gerbera hybrids and on one tomato cultivar (Moneymaker) at 20°C (after Dorsman and van de Vrie, 1987)

Gerbera hybrid	Percentage mortality	Developmental time (days)	Fecundity	rm
Clementine	16.1	34.0	234	0.080
Espace	15.2	34.8	274	0.084
Appelbloesem	10.0	32.4	195	0.083
Fame	13.4	32.2	249	0.087
Tomato	1.8	33.4	211	0.089

Whiteflies tested on gerbera were previously reared on gerbera.
Whiteflies tested on tomato came from the stock rearing on tomatoes from the Glasshouse Crops Research Station at Naaldwijk.

pelbloesem and Fame) did not differ: the rate of population development on these cultivars was similar (Table 5).

The next step was to qualify and quantify hairs on gerbera. A broad survey with 47 gerbera cultivars was made. From each cultivar 10 leafdiscs were taken to determine hair density and to measure hair length (Godthelp, 1989). Large differences, both in hair density and - type were found (see Fig. 4). The average hair density varied between 47.6 and 374.1 hairs per square centimeter. The average hair length varied between 0.81 and 2.00 millimeter. A greater hair density does not correlate with longer hairs.

A selection was made from these cultivars representing the diversity of hair lengths and densities. On these cultivars we first measured the walking speed of *E. formosa* (Fig. 5, data from van Lenteren et al., unpublished). From this figure it is clear that wasps walk slower when the hair density is larger, with the exception of cultivar

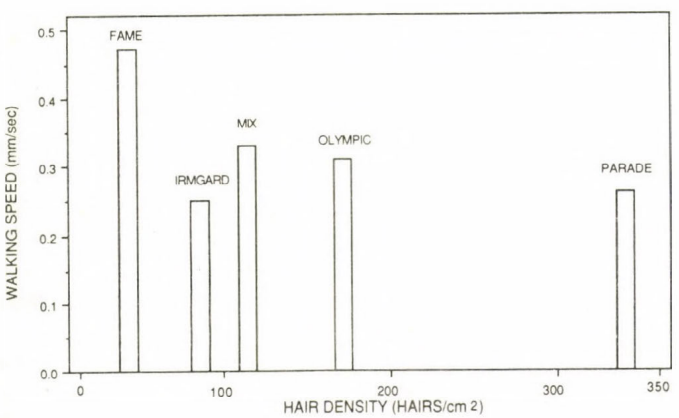

Fig. 5 Relationship between hair density on *Gerbera jamesonii* and walking speed by *Encarsia formosa* (P. W. T. Huisman and J. C. van Lenteren, unpubl.).

Table 6 Host-finding success and host-searching activity of *Encrasia formosa* on two gerbera cultivars in an arena on the leaf (after H. Godthelp and J. C. van Lenteren, unpublished).

Gerbera cultivar	% of wasps that found a host larva	Searching time till finding a host larva (in seconds)
Terra-Fame	97	1087
Parade	70	1179

Irmgard. The relatively low walking speed on leaves of cultivar Irmgard can easily be explained by the fact that the hairs are long and curled like a corkscrew, which is not found in the other cultivars. We intend to describe hair quality in more detail in the future and, further, not only to use hair density but percentage leaf cover by hairs as well to indicate more precisely which problems natural enemies might encounter.

In the following, preliminary experiment the cultivars Fame (53.5 hairs/cm^2 of 1.8 mm length) and Parade (266.7 hairs/cm^2 of 1.52 mm length) were used to assess the encounter probability and search interval between release and discovery of whitefly by *E. formosa*. The results are summarized in Table 6. Significantly more often whiteflies were found by *E. formosa* on the plants with the lower hair density. The average time needed to locate a whitefly on the two cultivars did not differ significantly. We plan to continue this work in order to find out under which conditions biological control of whitefly on gerbera is possible and how leaf hairiness interferes with this. In addition, we are presently studying the influence of hairiness of other flowering pot plants on the parasitization efficiency of *E. formosa*.

FINAL CONCLUSION

In order to be able to estimate the result of biological control of greenhouse whitefly with the parasite *E. formosa* on particular crops or hybrids, not only the developmental data of whitefly are necessary, but also information of leaf-surface qualities like hairs is of importance. Hairs may drastically impede the walking speed and change the walking pattern. These two factors may lead to a reduction in rate of parasitism. Further, long hairs retain droplets of honeydew and *E. formosa* can be "caught" and killed in these droplets, resulting in additional reduction in parasitism. Plant breeders should aim at selecting for hybrids with low hair densities in combination with selecting for (partial) whitefly resistance. The combined effect of biological control and partial resistance will lead to a relatively fast and long-term, environmentally safe whitefly control.

More generally we can conclude that there is a great need for concerted efforts of plant breeders and biological control workers in helping to find solutions for the presently very serious problems in crop protection. We see this as an important challenge for our respective professions!

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REFERENCES

Barbosa, P. and D. K. Letourneau (eds), (1988): *Novel Aspects of Insect-Plant Interactions*. Wiley, New York.

Bell, W. J. and R. T. Cardé (eds), (1984): *Chemical Ecology of Insects*. Chapman and Hall, London.

Berends, A. G. (1988): *Bestrijdingsmiddelen en oppervlaktewaterkwaliteit. Een inventarisatie van het gebruik van bestrijdingsmiddelen in de akkerbouw en tuinbouw*. Ministerie van Verkeer en Waterstaat, Dienst Binnenwateren RIZA, en Instituut voor Onderzoek van Bestrijdingsmiddelen, Lelystad.

Bergman, J. M. and W. M. Tingey (1979): Aspects of interactions between plant genotypes and biological control. *Bull. Entomol. Soc. Amer. 25*, 275-279.

Boethel, D.J. and R. D. Eikenbary (eds), (1986): *Interactions of Plant Resistance and Parasitoids and Predators of Insects*. Wiley, New York.

van Boxtel, W., J. Woets and J. C. van Lenteren (1978): Determination of host-plant quality of eggplant (*Solanum melongena* L.), cucumber (*Cucumis sativus* L.), tomato (*Lycopersicum esculentum* L.) and paprika (*Capsicum annuum* L.) for the greenhouse whitefly (*Trialeurodes vaporariorum* (Westwood)) (Homoptera: Aleyrodidae). *Mededelingen van de Faculteit der Landbouwwetenschappen, Rijksuniversiteit Gent, 43/2*, 397-408.

Colwell, A. E., H. H. Shorey, P. Baumer and S. E. van Vorhis Key (1978): Sex pheromone scent marking by females of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). *J. Chem. Ecol. 4*, 717-721.

Dicke, M. (1988): *Infochemicals in Tritrophic Interactions*. Ponsen en Looijen, Wageningen.

Dorsman, R. and M. van de Vrie (1987): Population dynamics of the greenhouse whitefly *Trialeurodes vaporariorum* on different gerbera varieties. *Bull. I.O.B.C./ W.P.R.S. X/2*, 46-51.

Elsey, K. D. (1974): Influence of the host on the searching behaviour of two predators. *Entomophaga 19*, 3-6.

van Emden, H. F. (1986): The interaction of plant resistance and natural enemies: Effects on populations of sucking insects. In: Boethel, D. J. and R. D. Eikenbary (eds), *Interactions of Plant Resistance and Parasitoids and Predators of Insects*. Wiley, New York. 138-150.

Godthelp, H. (1989): De bladbeharing van gerbera en de invloed hiervan op het zoekgedrag van *Encarsia. MSc. Thesis, University of Wageningen.*

Gurney, B. and N. W. Hussey (1970): Evaluation of some coccinellid species for the biocontrol of aphids in protected crops. *Ann. appl. Biol. 65*, 451-458.

Hoogcarspel, A. P. and J. A. Jobsen (1984): Laboratory method for testing side-effects of pesticides on *Encarsia formosa* Gahan (Hym., Aphelinidae): Results with pesticides used in glasshouse tomato crops in the Netherlands. *Z. ang. Entomol. 97*, 268-278.

Hulspas-Jordaan, P. M. (1977): Biologische bestrijding van de wittevlieg, *Trialeurodes vaporariorum* (Westwood)(Homoptera: Aleyrodidae) door de sluipwesp *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae): De invloed van de bladoppervlaktestructuur van de waardplant op de sluipwesp en het belang hiervan voor het bestrijdingssucces, toegespitst op een verbetering van het bestrijdingsresultaat op komkommer. *MSc Thesis, University of Leiden.*

Hulspas-Jordaan, P. M. and J. C. van Lenteren (1978): The relationship between host-plant leaf structure and parasitization efficiency of the parasitic wasp *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae). *Mededelingen van de Faculteit der Landbouwwetenschappen, Rijksuniversiteit Gent, 43/2*, 431-440.

Hulspas-Jordaan P.M. and J.C. van Lenteren (1989): The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). XXX. Modelling population growth of greenhouse whitefly on tomato. *Agricultural University Wageningen Papers* (in press)

Inggamer, H. and O. M. B. de Ponti (1983): Intermediary inheritance of glabrousness in cucumber. *Cuc. Genet. Coop. Rep. 6*, 24.

Janssen, J. A. M., W. F. Tjallingii and J. C. van Lenteren (1989): Electrical recording and ultrastructure of stylet penetration by the greenhouse whitefly. *Entomol. exp. appl. 52*, 69-81.

Jeffree, C. E. (1986): The cucticle, epicuticular waxes and trichomes of plants, with reference to their structure, functions and evolution. In: B. Juniper and R. Southwood (eds), *Insects and the Plant Surface*. Edward Arnold, London. 23-64.

Juniper, B. and R. Southwood (eds), (1986): *Insects and the Plant Surface*. Edward Arnold, London.

Kamerman, J. W. (1987): De biologische bestrijding van witte vlieg (*Trialeurodes vaporariorum* (Homoptera: Aleyrodidae)) met behulp van *Encarsia formosa* (Hymenoptera: Aphelinidae) op halfbehaarde komkommer. *MSc Thesis, University of Wageningen.*

van der Kamp, R. J. and J. C. van Lenteren (1981): The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). XI. Do mechanical barriers of the host plant prevent successful penetration of the phloem by whitefly larvae and adults? *Z. ang. Entomol. 92*, 149-159.

Kaneda, C. (1986): Interactions between resistant rice cultivars and natural enemies in relation to the population growth of the brown planthopper. In: D. J. Boethel and R. D. Eikenbary (eds), *Interactions of Plant Resistance and Parasitoids and Predators of Insects*. Wiley, New York. 117-124.

Kowalewski, E. and R. W. Robinson (1977): Whitefly resistance in *Cucumis. Bull. I.O.B.C./W.P.R.S. 1977/3*, 149-153.

van Lenteren, J. C. and L. P. J. J. Noldus (1989): Behavioural and ecological aspects of whitefly-plant relationships. In: D. Gerling (ed), *Whiteflies: Their Bionomics, Pest Status and Management*. Intercept Ltd., Wimborne, England. (in press)

van Lenteren, J. C. and J. Woets (1988): Biological and integrated pest control in greenhouses. *Ann. Rev. Entomol. 33*, 239-269.

van Lenteren, J. C., H. W. Nell, L. A. Sevenster-van der Lelie and J. Woets (1976): The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). I. Host finding by the parasite. *Entomol. exp. appl. 20*, 123-130.

van Lenteren, J. C., H. W. Nell and L. A. Sevenster-van der Lelie (1980): The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). IV. Oviposition behaviour of the parasite, with aspects of host selection, host discrimination and host feeding. *Z. ang. Entomol. 89*, 442-454.

van Lenteren, J. C., J. Woets, N. van der Poel, W. van Boxtel, S. van de Merendonk, R. van der Kamp, H. W. Nell and L. Sevenster-van der Lelie (1977): Biological control of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood)(Homoptera: Aleyrodidae) by *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) in Holland, an example of successfully applied ecological research. *Mededelingen van de Faculteit der Landbouwwetenschappen, Rijksuniversiteit Gent, 42/2*, 1333-1342.

van Lenteren, J. C., A. van Vianen, I. Hatala-Zseller and Cs. Budai (1989a): The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). XXIX. Suitability of two cultivars of sweet pepper, *Capsicum annuum* L., for two different strains of whiteflies. *J. appl. Entomol.* (in press)

van Lenteren, J. C., J. W. Kamerman, Z. H. Li and O. M. B. de Ponti (1989b): The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). Influence of hair density on parasitization and host feeding capacity of *Encarsia formosa* on cucumber. *J. appl. Entomol.* (submitted)

Li Zhao hua, F. Lammes, J. C. van Lenteren, P. W. Huisman, A. van Vianen and O. M. B. de Ponti (1987): The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). XXV. Influence of leaf structure on the searching activity of *Encarsia*. *J. appl. Entomol. 104*, 297-304.

Mound, L. A. (1965): Effect of leaf hair on cotton whitefly populations in the Sudan Gezira. *Empire Cotton Growing Review 42*, 33-40.

Mound L. A. and S. H. Halsey (1978): *Whitefly of the World. A Systematic Catalogue of the Aleyrodidae (Homoptera) with Host Plant and Natural Enemy Data*. Wiley and Sons, Chichester.

Noldus, L. P. J. J. (1989): Chemical espionage by parasitic wasps: how *Trichogramma* species exploit moth sex pheromone systems. *Ph.D. Thesis, University of Wageningen.*

Noldus, L. P. J. J. and J. C. van Lenteren (1989): Host-parasitoid interactions in a greenhouse ecosystem. In: M. Mackauer, L. E. Ehler and J. Roland (eds), *Critical Issues in Biological Control*. Intercept Ltd, Wimborne, England. (in press)

Noldus, L. P. J. J., Xu Rumei and J. C. van Lenteren (1986): The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). XIX. Feeding-site selection by the greenhouse whitefly. *J. appl. Entomol. 101*, 492-507.

Noldus, L. P. J. J., R. J. Potting and H. E. Barendregt (1989): Moth sex pheromone adsorption to leaf surface: bridge in time for chemical spies. *Physiol. Entomol.* (submitted)

Nordlund D. A., R. L. Jones and W. J. Lewis (eds), (1981): *Semiochemicals: Their Role in Pest Control*. Wiley, New York.

Obrycki, J. J., M. J. Tauber and W. M. Tingey (1983): Predator and parasitoid interaction with aphid resistant potatoes to reduce aphid-densities: a two year field study. *J. econ. Entomol. 76*, 456-462.

Obrycki, J. J. (1986): The influence of foliar pubescence on entomophagous species. In: D. J. Boethel and R. D. Eikenbary (eds), *Interactions of Plant Resistance and Parasitoids and Predators of Insects*. Wiley, New York. 61-83.

Ohnesorge, B., N. Sharaf and T. Allawi (1980): Population studies on the tobacco whitefly *Bemisia tabaci* Genn. (Homoptera; Aleyrodidae) during the winter season. I. Spatial distribution on some host plants. *Z. ang. Entomol. 90*, 226-232.

Papaj, D. R. and R. J. Prokopy (1989): Ecological and evolutionary aspects of learning in phytophagous insects. *Ann. Rev. Entomol. 34*, 315-350.

Pollard, D. G. and J. H. Saunders (1956): Relations of some cotton pests to jassid resistant sakel. *Empire Cotton Growing Review 33*, 197-202.

de Ponti, O. M. B. (1984): Recent developments of resistance to glasshouse whitefly in tomato and to two-spotted spider mites in cucumber. *Bull. O.I.L.B./S.R.O.P. VII/4*, 43-44.

Price, W. P. (1981): Semiochemicals in evolutionary time. In: D. A. Nordlund, R. L. Jones and W. J. Lewis (eds), *Semiochemicals: Their Role in Pest Control*. Wiley, New York. 251-279.

Price, W. P. (1986): Ecological aspects of host plant resistance and biological control: Interactions among three trophic levels. In: D. J. Boethel, and R. D. Eikenbary (eds), *Interactions of Plant Resistance and Parasitoids and Predators of Insects*. Wiley, New York. 11-30.

Rabb, R. L. and J. R. Bradley (1968): The influence of host plants on parasitism of the eggs of the tobacco budworm. *J. econ. Entomol. 61*, 1249-1252.

van Rongen, E. (1979): Biologische bestrijding van witte vlieg (*Trialeurodes vaporariorum*) op komkommer met de sluipwesp *Encarsia formosa*, Experimenten met onbehaarde komkommer. *MSc. Thesis, University of Leiden.*

Sabelis, M. W. and M. C. M. de Jong (1988): Should all plants recruit bodyguards? Conditions for a polymorphic EES of synomone production in plants. *Oikos 53*, 247-252.

Schuster, M. F. and M. Calderon (1986): Interactions of host plant resistant genotypes and beneficial insects in cotton ecosystems. In: D. J. Boethel and R. D. Eikenbary (eds), *Interactions of Plant Resistance and Parasitoids and Predators of Insects*. Wiley, New York. 84-97.

Singer, M. C. (1986): The definition and measurement of oviposition preference in plant-feeding insects. In: J. Miller and T. A. Miller (eds), *Insect-Plant Relations*. Springer, New York. 65-94.

Shorey, H. H. and J. M. McKelvey (eds), (1977): *Chemical Control of Insect Behavior: Theory and Application*. Wiley, New York.

Southwood, R. (1986): Plant surfaces and insects - an overview. In: Juniper, B. and R. Southwood (eds), *Insects and the Plant Surface*. Edward Arnold, London. 1-22.

Thompson, J. N. (1988): Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomol. exp. appl. 47*, 3-14.

van Venetie, R. (1978): De biologische bestrijding van witte vlieg (*Trialeurodes vaporariorum* (Westwood)) met behulp van de sluipwesp *Encarsia formosa* Gahan op haarloze komkommers in kassen. *MSc. Thesis, University of Leiden.*

van Vianen, A. and J. C. van Lenteren (1986): The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). XV. Oogenesis and oviposition of *Encarsia formosa*. *J. appl. Entomol. 101*, 130-139.

Wall, C. and J. N. Perry (1983): The behaviour of moths responding to pheromone sources in the fields: a basis for discussion. In: *Les Mediateurs Chimiques* Coll. INRA, No. 7. INRA, Paris. 171-188.

Willmer, P. (1986): Microclimatic effects on insects at the plant surface. In: Juniper, B. and R. Southwood (eds), *Insects and the Plant Surface*. Edward Arnold, London. 65-80.

Yano, E., J. C. van Lenteren, R. Rabbinge and P. M. Hulspas-Jordaan (1989): The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). XXXI. Simulation studies of the population growth of greenhouse whitefly on tomato. *Agricultural University Wageningen Papers*. (in press)

Yano, E., J. C. van Lenteren, R. Rabbinge, A. van Vianen and R. Dorsman (1989): The parasite-host relationship between *Encarsia formosa* (Hymonoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). XXXII. Simulation studies of the population growth of greenhouse whitefly on eggplant, cucumber, sweet pepper and gerbera. *Agricultural University Wageningen Papers*. (in press)

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ARE ATTRACTANT CHEMICALS ISOLATED FROM HOST-PLANTS OF PRACTICAL VALUE IN PEST CONTROL SYSTEMS? A HYPOTHESIS BASED ON EXPERIMENTS WITH THE CABBAGE ROOT FLY

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ABSTRACT

It is well established that the cabbage root fly (*Delia radicum* L.) is attracted to its host-plants by a wide range of naturally-occurring volatile isothiocyanates and related compounds. Many of these compounds have been isolated in quantities sufficient for field testing and one or two compounds, such as allylisothiocyanate, are readily-available and cheap. Allylisothiocyanate was used therefore as the main test chemical to determine whether such chemicals could prove useful in pest control under field conditions. It has been suggested that volatile host-plant chemicals, which at low doses attract pest insects to crop plants, can be used in pest control systems in one of four ways. They can be used at high doses either to 1) repel or 2) confuse the insects or they are used at lower doses in insect traps either 3) to directly reduce pest infestations or 4) to monitor pest-insect populations to decide the most appropriate times to apply insecticides.

The results of this paper describe experiments involving a range of volatile host-plant attractant chemicals under field conditions. The difficulties encountered in trying to employ such chemicals to assist in pest control in each of the four proposed ways are discussed. The results from the experiments carried out to date, indicate that it is questionable whether any benefits will accrue from using host-plant attractant chemicals, in any of the four ways suggested, in systems aimed at controlling cabbage root fly infestations in cruciferous vegetable crops.

Key words: cabbage root fly, *Delia radicum*, host-plant volatiles, allylisothiocyanate, attractants, water traps, chemical traps.

INTRODUCTION

The cabbage root fly (*Delia radicum* L.), referred to in North America as the cabbage maggot, is a member of the Anthomyiidae, or plant-feeding Muscidae, and is restricted to the temperate zone of the holarctic region (35°-60°N). Its larvae feed mainly on the roots, but sometimes on the aerial parts of cruciferous plants. It is a particularly destructive pest of cabbages and cauliflowers but it also attacks Brussels sprouts, radish, turnip, swede (rutabaga), garden stocks and many cruciferous weeds (Coaker and Finch, 1971).

The trap used currently at the Institute of Horticultural Research (IHR), Wellesbourne to capture adults of this and other cruciferous pest insects consists of a yellow water-trap fitted with a dispenser releasing allylisothiocyanate (ANCS) (Finch and Skinner, 1974). This odorous chemical is one of the naturally-occurring chemicals

that attracts cabbage root flies to their cruciferous host plants and, when added to a trap, considerably increases the numbers of flies caught (Finch and Skinner, 1982a). Experiments with other Orders of insects have indicated that, in addition to increasing the numbers of pest insects caught in traps, there are at least five other ways in which volatile chemicals could be used in pest control systems. The chemicals could be dispersed at high doses through crops to either repel the native insects or disrupt their normal mating behaviour. Lower doses could be used in traps to directly reduce pest numbers, indicate the most appropriate times to apply insecticides, or determine whether there are sufficient pest insects in a particular crop to warrant the application of insecticides.

This paper reviews research at IHR, Wellesbourne during the last 15 years on the response of the cabbage root fly to volatile host plant chemicals and highlights some of the difficulties encountered in trying to use volatile chemicals in pest control systems in the field.

EXPERIMENTAL

The usefulness of host plant chemicals in pest management schemes for the cabbage root fly is assessed in the following 5 Sections.

Throughout this paper, water traps without chemical dispensers are referred to as "standard traps" and those with ANCS dispensers as "ANCS traps".

1. Deterring adult cabbage root fly. Unlike sex pheromones, which are effective attractants when released from traps in nano- or micro-litre amounts, host-plant chemicals have to be released in millilitre amounts to be effective in traps (Finch, 1980). As a consequence, host-plant chemicals used in traps for the oriental fruit fly (*Dacus dorsalis* Hendel) (Steiner, 1952), the Japanese beetle (*Popillia japonica* Newman) (McGovern et al., 1970) and the cabbage root fly (Finch and Skinner, 1974) are currently released at rates of between 0.6-3 ml/trap per day.

It is surprising that such large amounts of volatile chemicals are required, as they greatly exceed the amounts released naturally by individual plants (Finch, 1980). McGovern et al. (1970) noted that many adults of *P. japonica* that approached traps failed to enter, and suggested that the concentration of the chemical vapour in the immediate vicinity of the trap might have been sufficiently high to be repellent. However, there was no indication that ANCS released from traps repelled the cabbage root fly, as standard traps placed in a 60 cm circle around a central ANCS trap failed to trap more flies than similar traps encircling a central standard-trap. It appears, therefore, that female cabbage root flies do not remain in the vicinity of ANCS traps without being caught. Other experiments showed that the numbers of flies caught remained unchanged when eight chemical dispensers were placed in the centre of individual traps, indicating that ANCS was not repellent to the cabbage root fly at release rates as high as 24 ml/trap per day from what was virtually a point source. It seems unlikely that deterring the cabbage root fly from entering crops by saturating the crops with host-plant chemical is likely to be practicable since, apart

from the high cost of the chemicals, most volatile chemicals released at these doses are extremely toxic to mammals.

2. Confusing adult cabbage root fly. Wright (1964) suggested the possibility of flooding an area with the sex attractant of females of a pest species to confuse the males and deprive them of directional information for finding the females. This approach has provided commercially-acceptable levels of control for a few insect species (see Roelofs and Cardé, 1977).

However, there seems little possibility, of confusing cabbage root fly in this manner, as similar numbers of root fly eggs were recovered from plants adjacent to traps releasing 24 ml ANCS/day as from plants outside the effective trap zones (Finch and Skinner, 1982a). By assuming that each plant released about 7 μl of volatile chemicals each day (Finch, 1980), it was estimated that each 24 ml trap released c. 180 times more chemical than did the crop plants into its 5 m trapping zone.

Similarly, attempts to confuse cabbage root flies by releasing 70 ml allylisothiocyanate/day through a 0.1 ha brassica plot failed to reduce the numbers of eggs laid (Finch, 1980). The difference in the responses of insects to host-plant volatiles and pheromones probably arises from the behaviour of flies within the host crop. Confusion strategies are likely to be of little use with flies attracted into the area of a host crop, as it is probable that the flies, whether confused or not, then alight on the plants and recognize suitable egg-laying sites through tarsal and gustatory stimuli.

3. Reducing cabbage root fly populations by trapping adults. To reduce insect populations adequately, traps releasing volatile chemicals must attract the target insects from at least tens of metres and retain a high percentage of individuals within their trapping zone (Hollingsworth and Hartstack, 1972).

Experiments have indicated that female cabbage root flies are distributed regularly through brassica crops (Finch and Skinner, 1973) and that the radius of the effective zone of the most effective trap tested to date was only c. 5 m. To increase the radius of the effective zone to 10 m, the distance suggested by Finch and Skinner (1974) as being required to assist in directly controlling this pest, the efficiency of the present ANCS trap must be increased at least four-fold, to overcome the present threshold in the numbers of females being caught by the present trap. This threshold seems likely to be due to the small proportion of the female fly population which at any one time is in the appropriate physiological state to respond to ANCS.

It seems unlikely that chemicals that will attract flies of all physiological states will be found, as naturally-occurring attractants extracted from the plant species most attractive to this fly were not more effective than ANCS in attracting flies to traps (Finch and Skinner, 1982b).

The main problem in trying to control insects directly with traps is how to deal with those situations where the new crop is not close to the site from which the insects emerge. In such situations, the flies mate "en route" and the females are ready to lay as soon as they enter the new crop. Few, if any, chemicals are able to attract "egg-bound" females away from their natural host-plants until the females have laid at least some of their eggs.

4. Monitoring changes in cabbage root fly populations. Generally it is assumed that, if the numbers of pest insects caught in a trap can be increased by releasing chemical from the trap, the chemical trap is better than the unbaited trap for monitoring changes in pest numbers. However, care is required in making assumptions of this type as traps used for pest monitoring schemes have to satisfy certain criteria. Thus, according to Moran (1951) the trap should not alter the probability of an insect being caught and the chance of being caught must remain similar for all individuals.

Comparisons made in 1982 on the relative effectiveness against cabbage root flies of standard and ANCS traps indicated that the ANCS trap satisfied neither criterion. The ANCS trap became more effective as the season progressed, rising from about 2.5x as effective as the standard trap in May to about 20x as effective by late September. As is usual with traps releasing volatile chemicals to attract day-flying insects, the numbers of cabbage root flies caught in the ANCS trap also increased disproportionately in periods of warm, settled weather. Similarly, the chance of being caught in ANCS traps did not remain equal for all individuals, as captures of females increased dramatically when the females had laid most of their eggs. As ANCS traps fail to satisfy the accepted criteria, they should not be used for monitoring changes in the population of this pest.

5. Threshold catches of the cabbage root fly. During 1982-1985, the numbers of cabbage root flies caught in standard traps and ANCS traps were correlated with the numbers of eggs recovered from the brassica crops in which the traps were sited. Means of c. 20 and 45 eggs/plant were recorded during the week of peak egg-laying by the first (May) and second (July) generations of cabbage root flies respectively. The numbers of flies caught in the standard traps averaged 28.3 and 60.5 for the two generations. Despite being the smaller of the two infestations, the first generation infestation severely damaged the roots of the crop whereas the second generation had no noticeable effect. Different "damage thresholds" are likely to be required for the two generations.

The relationship between the numbers of flies caught in the ANCS traps and the numbers of eggs recovered varied considerably between years. In addition, although large numbers of flies were caught in the ANCS traps during August and September (mean = 43/trap/day), few eggs were collected during the same period. Hence, the numbers of female cabbage root flies caught in the ANCS traps provided a poor indication of when insecticidal protection was needed by the crops. With the standard traps, most females were caught during the time of peak activity of the second generation in July (15/trap/day), and fewest (2/trap/day) in September when oviposition was low. Hence standard traps, rather than ANCS traps, gave the better indication of the periods when crops were most at risk from cabbage root fly attack.

CONCLUSIONS

Research at IHR Wellesbourne during the last 15 years has indicated that releasing large amounts of volatile host-plant chemicals into brassica crops is not likely to "deter" or "confuse" the cabbage root fly from causing damage in susceptible crops. Using volatile chemicals in traps to reduce pest numbers offers more promise, though large numbers of traps would be required and the outcome would often be unpredictable.

To date, traps releasing host-plant volatiles have not been shown to be suitable for monitoring changes in cabbage root fly populations. Further information is required on the effects of weather factors on trap chemicals and thus on the numbers of insects caught. Further difficulties remain in that the degree of overestimation of the sizes of fly populations by traps releasing host-plant volatiles cannot be predicted.

The major use of host-plant volatile chemicals with cabbage root fly is likely to be for increasing trap catches. However, it is not yet certain what the increased numbers of flies represent in terms of the absolute size of local populations. If existing non-chemical traps catch too few insects to provide an adequate estimate of the size of the insect population, this difficulty can probably be overcome most readily by using a greater number of traps or by altering the physical characteristics (shape, colour etc.) of the traps. With the limited knowledge available at present, it seems inadvisable to consider adding "attractants" to traps.

In general, the simpler the trap, the more robust it is likely to be as a tool in pest monitoring. Data for accurate cabbage root fly forecasting schemes are currently being obtained in the U.K. with non-chemical traps painted an appropriate colour (Finch and Collier, 1986).

REFERENCES

Coaker, T. H. and S. Finch (1971): The cabbage root fly *Erioischia brassicae* (Bouché). *Rep. natn. Veg. Res. Stn. for 1970,* 23-42.

Finch, S. (1980): Chemical attraction of plant-feeding insects to plants. *Appl. Biol. 5,* 67-143.

Finch, S. and R. H. Collier (1986): Forecasting the times of attack of the cabbage root fly (*Delia radicum*) on vegetable brassica crops. *Asp. Appl. Biol. 12,* 37-46.

Finch, S. and G. Skinner (1973): Distribution of cabbage root flies in brassica crops. *Ann. appl. Biol. 75,* 1-14.

Finch, S. and G. Skinner (1974): Some factors affecting the efficiency of water-traps for capturing cabbage root flies. *Ann. appl. Biol. 77,* 213-226.

Finch, S. and G. Skinner (1982a): Trapping female cabbage root flies (*Delia radicum* (L.)) (Diptera: Anthomyiidae) with allylisothicyanate-baited traps. *Bull. entomol. Res. 72,* 165-173.

Finch, S. and G. Skinner (1982b): Trapping cabbage root flies in traps baited with plant extracts and with natural and synthetic isothiocyanates. *Entomol. exp. appl. 31,* 133-139.

Hollingsworth, J. P. and A. W. Hartstack (1972): Effect of components on insect light trap performance. *Trans. Am. Soc. agric. Engrs. 15,* 924-927.

McGovern, T. P., M. Beroza, P. H. Schwartz, D. W. Hamilton, J. C. Ingangi and T. L. Ladd (1970): Methyl cyclohexanepropionate and related chemicals as attractants for the Japanese beetle. *J. econ. Entomol. 63,* 276-280.

Moran, P. A. P. (1951): A mathematical theory of animal trapping. *Biometrika 38,* 307-311.

Roelofs, W. L. and R. T. Cardé (1977): Responses of Lepidoptera to synthetic sex pheromone chemicals and their analogues. *Ann. Rev. Entomol. 22,* 377-405.

Steiner, L. F. (1952): Methyl eugenol as an attractant for oriental fruit fly. *J. econ. Entomol. 45,* 241-248.

Wright, R. H. (1964): *The Science of Smell.* George Allen and Unwin Ltd., London.

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TRITROPHIC INTERACTIONS BETWEEN GRAINS, THE GREENBUG (*SCHIZAPHIS GRAMINUM* RONDANI) AND ENTOMOPHAGA

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ABSTRACT

Two important cereal aphids, the greenbug, *Schizaphis graminum* (Rondani), and the Russian wheat aphid, (RWA) *Diuraphis noxia* Mordvilko, seriously limit grain production in the central and western North American continent. Greenbug populations consist of several different biotypes based upon differential abilities to damage cereal resistance sources. The most important in the field being biotypes C (GBC) and E (GBE). Effective resistance sources for RWA have not yet been located in wheat and barley, its major hosts. The studies reported here were designed to clarify tritrophic effects of primarily antibiotic or tolerant resistance sources in barley, grain sorghum, and wheat interacting with GBC, GBE, or RWA and the most prevalent cereal aphid parasitoid in the region, *Lysiphlebus testaceipes* (Cresson).

Key words: wheat, *Triticum aestivum*, grain, sorghum, *Sorghum bicolor*, barley, *Hordeum vulgare*, greenbug, *Schizaphis graminum*, Russian wheat aphid, *Diuraphis noxia*, *Lysiphlebus testaceipes*, biological control, host-plant resistance, root biomass, stem biomass.

INTRODUCTION

The greenbug, *Schizaphis graminum* (Rondani), has long been considered one of the most injurious insect pests attacking grains in the United States. This aphid has been known to possess a wide host range (Pettersson, 1971), but in recent years has been expanding its adaptive range to colonize both new host species such as grain sorghum (Harvey and Hackerott, 1969) as well as formerly resistant crop cultivars developed in germplasm enhancement programs. A number of greenbug biotypes have been defined based on their differential abilities to damage greenbug resistance sources in wheat, sorghum, oats, rye, and barley (see Puterka et al., 1988). The most agronomically important and prevalent in the field being greenbug biotypes C (GBC) and E (GBE) (Bush et al., 1987; Kerns et al., 1987).

The parasitoid *Lysiphlebus testaceipes* (Cresson) is the most abundant and recurrent natural enemy of the greenbug in the U.S. (Jackson et al., 1970; Archer et al. 1974). It has also been shown to complement host plant resistance (HPR) in the field (Hamilton et al., 1982).

The Russian Wheat Aphid (RWA), *Diuraphis noxia* Mordvilko is a new and serious pest of small grains in the U.S. first found in Texas in 1986 (Stoetzel, 1987).

L. testaceipes can parasitize RWA in the field (Morrison, 1988), yet laboratory studies indicate that it greatly prefers the greenbug (Gilstrap and McKinnon, 1988). Commonly used wheat varieties, including those resistant to certain greenbug biotypes, do not possess significant levels of resistance to the RWA (Webster et al., 1987) though some resistance sources are available in lines of triticale (Frank et al., 1989).

HPR and biological control (BC) are often considered compatible pest management strategies (Bergman and Tingey, 1979; see Boethel and Eikenbary, 1986). When both control methodologies are used concurrently, unrelated mortality effects are applied, which reduces the rate of genetic adaptation in the pest population to selection pressures imposed by resistant germplasms, thus possibly slowing the development of new pest biotypes. However, parasitoid performance may be detrimentally altered by the host plant of the pest insect if resistance is based upon significant chemical antibiosis. Although different species of host plant can produce the greatest range in responses, cultivars of the same species can also differentially affect parasitoid success (de Ponti, 1980). Furthermore, four trophic level interactions (e.g. hyperparasitoids) must also be considered (Orr and Boethel, 1986).

Starks et al. (1972) found resistant varieties of barley and sorghum to be complementary to the effect of *L. testaceipes* in laboratory studies, although the effect was not evident in caged field studies using sorghum (Starks et al., 1974). Schuster and Starks (1975) showed the response of *L. testaceipes* was improved in a resistant variety of oats over a susceptible one, but again no differences occurred with sorghum. Salto et al. (1983) found no differences in the parasitization of greenbug on resistant and susceptible oats. Kuo (1986) concluded that oat plant resistance against cereal aphids and the effect of an aphid parasitoid seem to complement each other in reducing the number of aphids but emphasized the importance of studies on the fecundity and sex ratio of the parasitoids. We are unaware of any similar studies conducted on resistant and susceptible wheat varieties. The research reported herein was designed to further investigate such tritrophic interactions using wheat, barley and sorghum; the aphids GBC, GBE and RWA; and *L. testaceipes*.

MATERIALS AND METHODS

Both studies reported here were performed in controlled environmental chambers and used recently collected insect colonies that were maintained in a greenhouse or chambers. Chamber relative humidity was not controlled and ranged from 60-70%.

Experiment 1. This experiment was designed to compare the effects to *L. testaceipes* of a primarily antibiotic resistance source in barley (cv. 'Post') and a susceptible variety ('Wintermalt') using GBE as the aphid. It also compared a primarily tolerant resistance source in sorghum (cv. 'Capbam') to a susceptible (cv. 'Pioneer 8300') using the same aphid. Chamber temperature was set at 24°C during the 13 hour photophase and at 20°C during the scotophase. GBE colonies were maintained on each barley variety for several generations prior to the beginning of the experiment, after which parasitoid life history and reproductive responses were monitored

Table 1 L. testaceipes Parental generations - Barley

Character measured	Susteptible 'Wintermalt'	Resistant 'Post'
Parasitization* success (%)	87.3±5.2	67.9±5.3
Days to 1st mummy*	6.7±0.1	7.3±0.1
Period over which mummies formed (days)*	2.8±0.2	3.5±0.2
Max. mummies/days/females*	14.2±0.8	3.4±0.9
Day number of maximum mummies*	7.4±0.2	8.1±0.2
Total mummies/females*	20.0±1.3	6.8±1.5

* All characters significantly different at $P \leq 0.05$ by t-test. Values are means ± standard errors.

through five successive generations. Each parasitoid mating pair were offered an excess of GBE for oviposition on two plants in a caged pot. Ten replications were performed per generation. Each day all mummies were removed and counted and a maximum of five from each pot were retained for subsequent size and developmental rate measurements.

Experiment 2. Experiment 2 was designed to compare the interacting effects of GBC, GBE, or RWA and *L. testaceipes* when reared on wheat varieties either resistant to GBC (cv. 'TAM-107') or susceptible to it (cv. 'TAM-105'). TAM-107 is expected to exhibit little or no resistance to GBE and RWA. Experiments were performed in growth chambers at a constant 22°C and a 14 hour photophase. The system consisted of one caged plant (GS 11-12, 14 days old) upon which one aphid

Table 2 L. testaceipes F_1 adult subsample - Barley

Character measured	Susceptible 'Wintermalt'	Resistant 'Post'
Mummy width (μm)*	862±7	735±9
Head capsule width μm)*	376±3	344±3
Femur length μm)*	276±3	241±3
Development time* (mummy-adult, days)	3.96±0.06	4.35±0.08

* All characters significantly different at $P \leq 0.05$ by t-test. Values are means ± standard errors.

nymph less than one day old was placed. This was considered Day 0. Those treatments that included the parasitoid effect received one mating pair of *L. testaceipes* on Day 8. The experiment was a lattice design with five replicates and was analyzed as a factorial experiment. Aphid population growth was monitored until Day 30 or the day upon which the plant was declared dead. Also at this time, the dried root and stem biomasses were obtained.

RESULTS AND DISCUSSION

Experiment 1. Table 1 indicates reproductive performance parameters for the parasitoid reared on GBE on the two barley varieties. All parameters were signifi-

Table 3 L. testaceipes Parental generations - Sorghum

Character measured	Susteptible 'Pioneer-8300'	Resistant 'Capbam'
Parasitization success (%)	91.3±5.2	94.0±5.1
Days to 1st mummy	6.6±0.1	6.9±0.1
Period over which mummies formed (days)*	3.5±0.2	5.1±0.2
Max. mummies/days/females*	11.4±0.8	7.2±0.8
Day number of maximum mummies*	7.4±0.2	8.4±0.2
Total mummies/females	20.3±1.2	19.3±1.2

* Characters marked are significantly different at $P \leq 0.05$ by t-test. Values are means ± standard errors.

cantly influenced in a detrimental manner by the variety expressing antibiosis. Table 2 indicates mummy and adult size measurements and developmental times from the subsample of mummies reared on the barley varieties. Again, all parameters were detrimentally affected by the 'Post' variety.

Table 3 indicates reproductive performance parameters for the parasitoid reared on GBE on the two sorghum varieties. Here, the parasitization success (% of females producing mummies), the days to the formation of the first mummy, and the total number of mummies produced per female were not detrimentally affected by the tolerant resistance source 'Capbam'. Table 4 indicates the size and developmental time measurements for the mummies reared on the sorghum varieties. All of these factors were detrimentally affected.

The results of Experiment 1 illustrate the severe effects of a strong chemical antibiosis in 'Post' barley to *L. testaceipes*. While the tolerant sorghum appears to have less detrimental effects it still causes smaller aphid mummies and parasitoid adults which are likely weaker and that have extended developmental times. It appears evident that the use of resistant plant varieties based primarily upon antibiosis can greatly limit the capabilities of a parasitoid that might otherwise provide substantial benefit. Such interactions should be carefully investigated on a case by case basis by entomologists and plant breeders.

Experiment 2. Fig. 1 illustrates population growth curves for GBC on resistant ('TAM-107') and susceptible ('TAM-105') wheat varieties with and without the influence of *L. testaceipes*. It can be seen that the resistant wheat alone extended plant survival from 18 to 28 days but allowed a substantial buildup in aphid numbers. Comparing the susceptibles, parasitoid presence extended plant survival but did not

Table 4 L. testaceipes F_1 adult subsample - Sorghum

Character measured	Susceptible 'Pioneer-8300'	Resistant 'Capmam'
Mummy width (μm)*	843±7	728±7
Head capsule width μm)*	369±3	329±2
Femur length μm)*	271±2	236±2
Development time* (mummy-adult, days)	3.98±0.06	4.27±0.06

* All characters significantly different at $P \leq 0.05$ by t-test. Values are means ± standard errors.

greatly limit peak aphid numbers. The combination of resistant wheat and parasitoid greatly limited aphid numbers and likely would have driven the aphids to extinction in the closed system had the experiment not been terminated on Day 30. Fig. 2 indicates that 'TAM-107' resistance to GBE extended plant survival 6 days. The parasitoid in combination with 'TAM-107' extended plant survival 4 additional days.

Russian wheat aphid population numbers are presented in Fig. 3. It is evident from inspection of these curves that neither the 'TAM-107' resistance source nor the presence of *L. testaceipes* is capable of effective population suppression. The resistance source only slightly retards population growth and the parasitoid was noted to only rarely oviposit in RWA, thus supporting the results of Gilstrap and McKinnon (1988).

Table 5 provides dried plant biomass values for the various treatment combinations in Experiment 2. The 'TAM107' wheat cultivar is generally considered to be more robust than 'TAM105' and this is born out by these values. The RWA, in particular, seriously limits root biomass production. With the greenbug, parasitoid presence or plant resistance may help to preserve biomass productivity. Here again, however, no helpful parasitoid effect can be seen with RWA.

Unfortunately, the most common greenbug resistance source in wheat and the most common cereal aphid parasitoid in the U.S. are ineffective against the RWA. More effective resistance sources must be introduced into commercial cereal var-

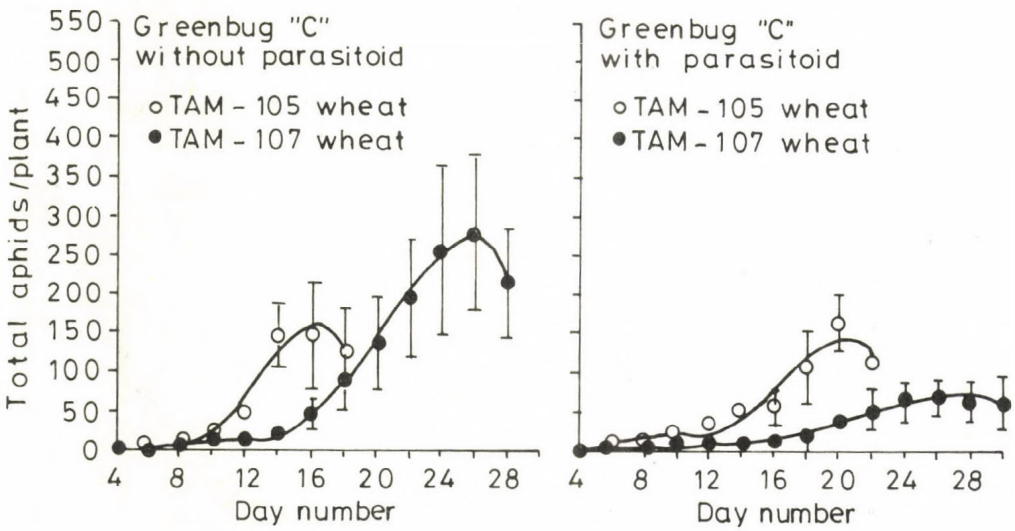

Fig. 1 Populations of greenbug biotype C with and without *L. testaceipes* introduced on Day 8. 'TAM-105' susceptible, 'TAM-107' resistant. Means ± SE.

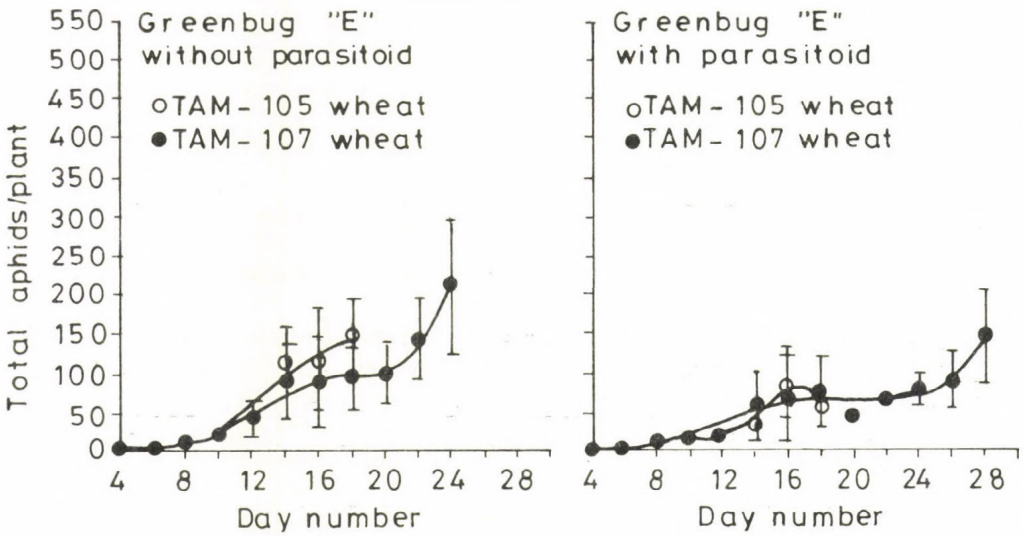

Fig. 2 Populations of greenbug biotype E with and without *L. testaceipes* introduced on Day 8. Otherwise same as Fig. 1.

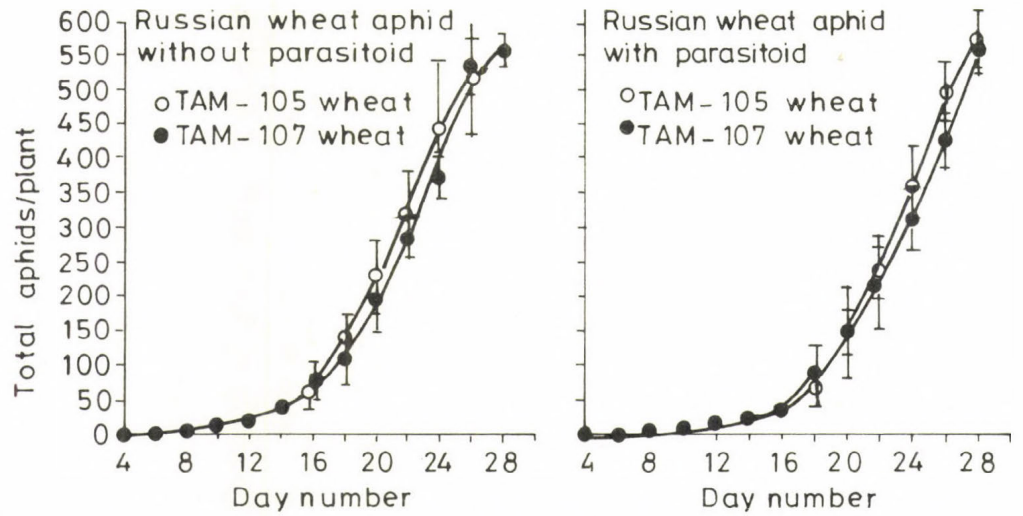

Fig. 3 Populations of Russian wheat aphid with and without *L. testaceipes* introduced Day 8. Otherwise like Fig. 1.

Table 5 Plant biomass values on day of death

Wheat Variety	Wheat Variety			
Treatment	'TAM-105'		'TAM-107'	
Root Wt.	Root Wt.	Stem wt.	Root wt.	Stem wt.
Control	38±10	92±16	52±11	107±15
GBC	14±2	36±7	36±11	72±16
GBC - P	12±2	36±8	40±7	110±12
GBE	12±2	36±8	28±7	54±14
GBE - P	20±6	54±18	40±11	106±21
RWA	14±4	46±7	16±2	62±11
RWA - P	10±2	46±6	18±5	58±4

Values are means of five replicates ± standard errors and are in milligrams dried weight. Control = no aphid; aphid designations followed by "P" indicate parasitoid introduced day 8.

ieties, more capable parasitoids must be located and established in the U.S., and the interactions of these control approaches should be carefully analyzed both in the laboratory and under field conditions.

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REFERENCES

Archer, T. L., R. H. Cate, R. D. Eikenbary, and K. J. Starks (1974): Parasitoids collected from greenbugs and corn leaf aphids in Oklahoma in 1972. *Ann. Entomol. Soc. Amer. 67*, 11-14.

Boethel, D. J. and R. D. Eikenbary (1986): *Interactions of Plant Resistance and Parasitoids and Predators of Insects.* Ellis Horwood Ltd., Chichester.

Bergman, J. M. and W. M. Tingey (1979): Aspects of interaction between plant genotypes and biological control. *Bull. Entomol. Soc. Amer. 25*, 275-279.

Bush, L., J. E. Slosser, W. D. Worrall, and G. J. Puterka (1987): Status of greenbug biotypes in Texas. *Southwest. Entomol. 12*, 229-235.

Frank, W. A., R. A. Scott, J. E. Slosser, and W. D. Worrall (1989): Resistance in triticale to Russian wheat aphid and greenbug. *Southwest. Entomol. 14*, 167-178.

Gilstrap, F. E. and L. K. McKinnon (1988): Response of native parasites to Russian wheat aphid. *Texas Agr. Exp. Sta. PR-4558.* 5.

Hamilton, G. C., R. L. Kirkland, and I. D. R. Peries (1982): Population ecology of *Schizaphis graminum* (Rond.) (Homoptera: Aphididae) on grain sorghum in central Missouri. *Environ. Entomol. 11*, 618-628.

Harvey, J. L. and H. L. Hackerott (1969): Recognition of a greenbug biotype injurious to sorghum. *J. econ. entomol. 62*, 776-779.

Jackson, H. B., L. W. Coles, E. A. Wood, Jr. and R. D. Eikenbary (1970): Parasites reared from the greenbug and corn leaf aphid in Oklahoma in 1968 and 1969. *J. econ. entomol. 63*, 733-736.

Kerns, D. L., D. C. Peters and G. J. Puterka (1987): Greenbug biotype and grain sorghum seed sale surveys in Oklahoma, 1986. *Southwest. Entomol. 12*, 237-243.

Kuo, H. L. (1986): Resistance of oats to cereal aphids: Effects on parasitism by *Aphelinus asychis* (Walker). In: D. J. Boethel and R. D. Eikenbary (eds), *Interactions of Plant Resistance and Parasitoids and Predators of Insects*. Ellis Horwood Ltd., Chichester.

Morrison, P. (1988): The Russian wheat aphid. In: S. Coppock and B. Massey (eds), *Proc. of the First Russian Wheat Aphid Conference*. Coop. Ext. Serv. Bull. E-875, Okla. State Univ., Stillwater.

Orr, D. B. and D. J. Boethel (1986): Influence of plant antibiosis through four trophic levels. *Oecologia 70*, 242-249.

Pettersson, J. (1971): Studies on four grass-inhabiting species of *Schizaphis* (Homoptera: Aphididae). III. Host Plants. *Swed. J. Agr. Res. 1*, 133-138.

de Ponti, O. M. B. (1980): Breeding glabrous cucumber (*Cucumis sativus*) varieties to improve the biological control of the greenhouse whitefly (*Trialeurodes vaporariorum*). In: A. K. Minks and P. Gruys (eds.), *Integrated Control of Insect Pests in The Netherlands*. Pudoc, Wageningen.

Puterka, G. J., D. C. Peters, D. L. Kerns, J. E. Slosser, L. Bush, D. W. Worrall and R. W. McNew (1988): Designation of two new greenbug (Homoptera: Aphididae) biotypes G and H. *J. econ. Entomol. 81*, 1754-1759.

Salto, C. E., R. D. Eikenbary and K. J. Starks (1983): Compatibility of *Lysiphlebus testaceipes* (Hymenoptera: Braconidae) with greenbug (Homoptera: Aphididae) biotypes C and E reared on susceptible and resistant oat varieties. *Environ. Entomol. 12*, 603-604.

Schuster, D. J. and K. J. Starks (1975): Preference of *Lysiphlebus testaceipes* for greenbug resistant and susceptible small grain species. *Environ. Entomol. 4*, 887-888.

Starks, K. J., R. Muniappan and R. D. Eikenbary (1972): Interaction between plant resistance and parasitism against the greenbug on barley and sorghum. *Ann. Entomol. Soc. Amer. 65*, 650-655.

Starks, K. J., E. A. Wood, Jr. and R. L. Burton (1974): Relationships of plant resistance and *Lysiphlebus testaceipes* to population levels of the greenbug on grain sorghum. *Environ. Entomol. 3*, 950-952.

Stoetzel, M. B. (1987): Information on and identification of *Diuraphis noxia* (Homoptera: Aphididae) and other aphid species colonizing leaves of wheat and barley in the United States. *J. econ. Entomol. 80*, 696-704.

Webster, J. A., K. J. Starks and R. L. Burton (1987): Plant resistance studies with *Diuraphis noxia* (Homoptera: Aphididae), a new United States wheat pest. *J. econ. Entomol. 80*, 944-949.

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INFLUENCE OF *PHYLLOXERA NOTABILIS* PERGANDE ON *CYDIA CARYANA* (FITCH) POPULATIONS ON *CARYA ILLINOENSIS* (WANG) GENOTYPES

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ABSTRACT

Pecan, *Carya illinoensis* (Wang), genotypes influence population levels of the hickory shuckworm, *Cydia caryana* (Fitch), (HSW). For instance, the infestation level of galls on 400 pecan leaves sampled at random cultivars of "Oklahoma", "Mahan" and "Schley" were 5247, 1553, and 227. The percentage of these galls infested with HSW were 15.7, 24.1 and 11.0, respectively. Also, phenotypes of the cultivar "Oklahoma" varied considerably as to their susceptibility to attack from *Phylloxera notabilis* Pergande and there were distinct differences in the two groups (low or high gall phenotypes). The low gall "Oklahoma" phenotype did not have as high percentage of HSW larvae as the high gall phenotypes. The HSW pheromone trap catch followed the same trend for high and low *notabilis* phenotype trees. However, there were subtle differences in HSW trap catch in high and low gall phenotypes in different years. Galls appeared to be of greater importance as ovipositional sites for propagating HSW species during years of fewer nuts on the tree than during years of heavy nut set when there were many ovipositional sites available.

Key words: *Carya illinoensis*, *Cydia caryana*, galls, hickory, hickory shuckworm, "Oklahoma" cultivar, *Phylloxera devastatrix*, *P. notabilis*, pecan leaf phylloxera, pecan stem phylloxera.

INTRODUCTION

The hickory shuckworm, *Cydia caryana* (Fitch) (Lepidoptera: Tortricidae) (HSW), is a serious pest that infests pecans, *Carya illinoensis* (Wang) (Walker, 1933; Payne and Heaton, 1975). The host range of HSW is similar to the host range of *C. illinoensis* over the eastern portion of the United States (Osburn et al., 1963; Hall, 1983).

The HSW overwinters as mature larvae in the shuck or husk of the pecan, which may be either on the ground or on the tree (Moznette, 1941). The adults emerge from February through November with peaks occurring in May, July and August-September (Hall, 1983). Calcote and Hyder (1979, 1980) reported that the overwintering generation emerge in a bimodal pattern and as late as July, August and September. Oviposition occurs on the nuts of pecan or other hickory species and on the galls of the pecan stem phylloxera, *Phylloxera devastatrix* Pergande, or the leaf phylloxerae, *Phylloxera notabilis* Pergande and *Phylloxera russellae* Stoetzel (Moznette et al., 1931; Walker, 1933; Welch and Van Cleave, 1967; Boethel et al., 1974,

1979; Boethel and Ezell, 1977; Calcote and Hyder, 1980; Mitchell et al., 1984; and Dinkins and Reid, 1983, 1988).

HSW damage can be categorized into several types depending on the phenology of the pecan (Hall, 1983). Prior to shell hardening, the HSW larvae feed on the interior of the nut. This feeding results in nut abortion (Moznette et al., 1931; Walker, 1933; Tedders and Edwards, 1970; Payne and Heaton, 1975). After shell hardening, the larvae feed within the shuck and cause several types of damage including shuck adherence to the shell, scarring, discoloration of the shell, and poor kernel formation (Tedders and Edwards, 1970; Payne and Heaton, 1975; Hall, 1983, Calcote, 1989).

Pecan genotypes influence population levels of the HSW. Welch and Van Cleave (1967) and Hansen et al. (1970) found the cultivars "Moore" and "Desirable" to have significantly less damage than "Mahan" and "Stuart". Neel (1959) quantified infestation levels of four genotypes and found "Success" to have the highest infestation rate followed by "Stuart", "Pabst" and "Schley". Calcote et al. (1976, 1979) reported that the "Cape Fear" and "Gra-Bohls" genotypes and progeny having the "Evers" genotype as a staminate parent had lower infestation levels. Selections having "Burkett" or "Carmichael" as staminate parents or "Success", "Mahan", "Mohawk", or "Halbert" as pistillate parents had higher infestation rates. According to Sparks (1977) "Stuart" is one of the most susceptible cultivars to the HSW. Johnston (1989) reported that the number of HSW larvae per nut in the shucks vary by susceptible cultivar.

Population levels of phylloxera are influenced by pecan genotypes. For instance, Boethel et al. (1974) reported the infestion of level of galls on 400 pecan leaves sampled at random on cultivars of "Oklahoma", "Mahan" and "Schley" as 5247, 1553 and 227. The percentage of these galls infested with HSW were 15.7, 24.1 and 11.0, respectively. The incidence of hickory shuckworm in galls of the pecan leaf phylloxera ranged up to 22% and for the pecan stem phylloxera up to 89% for some *C. illinoensis* genotypes in certain years (Boethel et al., 1974). Dinkins and Reid (1988) found variation in phylloxera infestations on seedlings genotypes. Additionally, this research demonstrated that it may take more than one phylloxera gall for the shuckworm larvae to complete development. Therefore, Boethel et al. (1974) concluded that phylloxera galls could be responsible for maintaining endemic levels of HSW in or near pecan orchards.

Entomologists in the past have evaluated phylloxera and HSW populations separately or partially through the season, probably because there was not a practical method of monitoring HSW populations on individual trees until the development of the hickory shuckworm sex pheromone (Smith et al., 1987, McDonough et al., in press). HSW male populations can now be monitored in each tree throughout the season using pheromone baited traps.

This study evaluates the influence of *P. notabilis* galls on HSW populations in "Oklahoma" genotype pecans exhibiting different phenotypic expression levels of *notabilis* infestation susceptibility.

MATERIALS AND METHODS

We think a large population of galls could affect the HSW population in the trees. For our research, three samples of galls were taken and examined for evidence of HSW. These studies were taken in October 1988, 15 May and 15 June 1989.

The gall samples were taken from a single cultivar of trees ("Oklahoma"). The sample consisted of a collection of 100 galls per tree, if 100 galls had not been found from the leaf collection, the sample would stop at 100 leaves. Once these leaves had been collected, the galls were counted and inspected in order to find HSW larvae or evidence of larvae. After data collection, the trees were divided into two groups based on phenotypic expression of high or low susceptibility to gall infestations.

RESULTS

Phenotypes varied considerably as to their susceptibility to attack from *notabilis* and there were distinct differences in the two groups (low or high gall phenotypes). The HSW larvae appeared to infest more galls as the season progressed and then diminished as the galls became unsuitable at the end of June and/or early July. The low gall phenotypes did not appear to accumulate as high a percentage of HSW larvae as the high gall phenotypes.

The mean number of galls per 100 leaves were 20, 50 and 150 for high gall genotypes and 0, 2 and 2 for low gall phenotypes for sampling dates of 10-88, 5-89, and 6-89, respectively. The percentage of these galls that showed evidence (fecal material) or had HSW larvae for the high gall phenotypes was 45, 16 and 79 and for the low gall phenotypes 5, 44 and 30 for these sampling dates.

In general, the HSW pheromone trap catch followed the same trend for high and low *notabilis* phenotype trees (Fig. 1-4). When few moths were trapped during a sample date in the high gall phenotypes, there were few moths trapped in the low gall phenotypes. Also, if there were many HSW trapped in the high gall phenotypes,

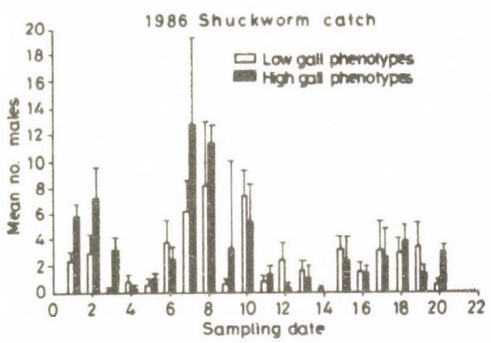

Fig. 1 Mean number of HSW males caught in pheromone traps in low and high gall phenotypes in 1986.

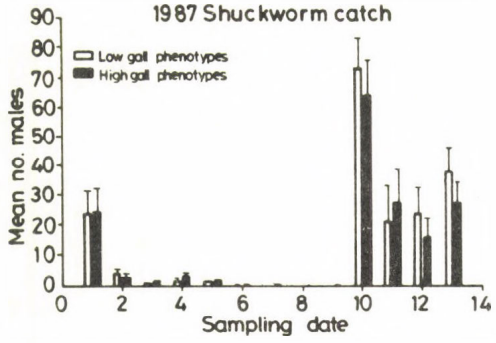

Fig. 2 Mean number of HSW males caught in pheromone traps in low and high gall phenotypes in 1987.

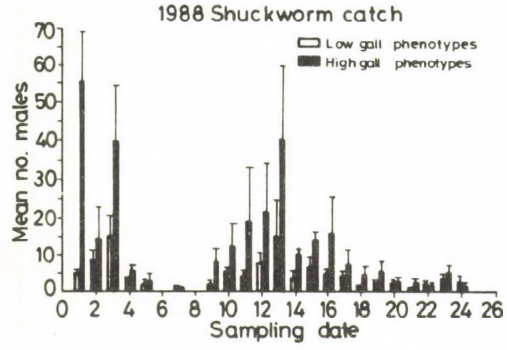

Fig. 3 Mean number of HSW males caught in pheromone traps/tree in low and high gall phenotypes in 1988.

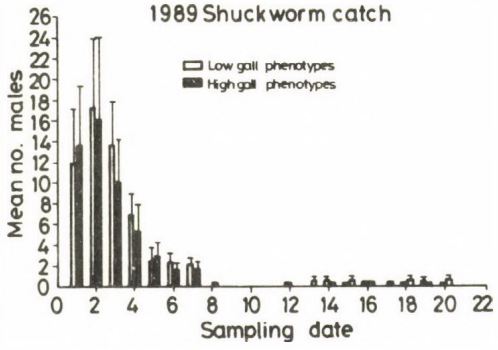

Fig. 4 Mean number of HSW males caught in one pheromone trap/tree in low and high gall phenotypes in 1989.

there were many moths trapped in the low gall phenotypes. However, there were subtle differences in HSW trap catch in high and low gall phenotypes in different years. In 1986 and 1988, high gall phenotype trees had a higher trap catch of HSW than low gall phenotype trees (Fig. 1 and 3). However, trees of low gall phenotype had an approximately equal catch of HSW as high gall phenotype trees in 1987 and 1989 (Figs. 2 and 4).

The difference in pheromone trap catch between high and low phenotypes in 1986 and 1988 compared to the 1987 and 1989 HSW trap catch may be the result of the alternate bearing of these trees. During years of high nut set on the trees (1986 and 1988), there were many ovipositional sites available, but during the years 1987 and 1989, there was a low nut set on the trees and fewer ovipositional sites. Therefore, galls may be of greater importance as ovipositional sites for propagating HSW species during off crop years when there are fewer nuts on the tree than during years of heavy nut set when there are many ovipositional sites available.

DISCUSSION

Some *C. illinoensis* genotypes are more susceptible to the hickory shuckworm and *Phylloxera* spp. than other cultivars. Where *C. illinoensis* genotypes are susceptible to both of these pests, large HSW populations can be expected.

Phylloxera spp. can provide an early season habitat for HSW development from approximately early May to early July. The HSW that develop from these galls attack other galls or the pecan nuts to perpetuate the HSW species. Pecans are available for oviposition by HSW from about 1 June to 15 October. HSW larvae that feed on nuts up until the end of June have sometimes been considered to be suicidal by pecan entomologists. However, Calcote (USDA-ARS, Stoneville, Mississippi, personal communication, 1989) and Hall (Pecan Research Experiment Station, Shreveport, Louisiana, personal communication, 1989) have reared HSW moths from aborted nuts after early July. Since some pecan genotypes start maturing (shell hardening) early, HSW can emerge from *Phylloxera* spp. galls in early July and attack nuts on early maturing genotypes, thus allowing HSW to develop to maturity. This would provide an endemic HSW population through the summer (Boethel et al., 1974) in addition to those HSW that develop in aborted nuts and moths that might emerge from the overwintering shucks (Calcote, personal communication, 1989). Apparently, *Phylloxera* spp. have co-evolved with HSW and this has resulted in the large emergence of HSW populations early in the season prior to the appearance of suitable nuts for development of HSW larvae.

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REFERENCES

Boethel, D. J. and J. E. Ezell (1977): Pecan *Phylloxera* pictorial life history. *Pecan South 4*, 52-55.

Boethel, D. J., J. T. Criswell and R. D. Eikenbary (1974): Incidence of hickory shuckworm in galls of *Phylloxera* spp. on pecans. *J. Econ. Entomol. 67*, 692-693.

Boethel, D. J., J. M. McBride and J. E. Ezell (1977): 1976 Louisiana insect control studies. *Pecan South 4*, 77-79.

Boethel, D. J., R. R. Shelton and J. M McBride (1979): *Phylloxera* control studies. *Pecan South 6*, 14-16.

Calcote, V. R. (1989): Seasonal occurrence of the hickory shuckworm in pecan. *Proc. S. E. Pecan Growers Assoc. 82*, 55-62.

Calcote, V. R. and D. E. Hyder (1979): Occurrence of a bimodal emergence pattern in the hickory shuckworm. *J. Econ. Entomol. 72*, 701-702.

Calcote, V. R. and D. E. Hyder (1980): Late season emergence of shuckworm from overwintering shucks. *Proc. S. E. Pecan Growers Assoc. 73*, 75-77.

Calcote, V. R., G. D. Madden and H. D. Patterson (1976): Pecan cultivars resistant to hickory shuckworm. *Ann. Rep. Northern Nut Growers Assoc. 67*, 19-21.

Calcote, V. R., G. D. Madden and H. D. Petterson (1979): Pecan cultivars tested for resistance to hickory shuckworm. *Pecan Quarterly 11*, 4-5.

Dinkins, R. L. and W. R. Reid (1983): Gall-forming stages of the stem phylloxera. *Phylloxera devastatrix* Pergande, on Kansas pecan trees, with notes on new state records of phylloxeran species distribution. *J. Kansas Entomol. Soc. 56*, 452.

Dinkins, R. L. and W. R. Reid (1988): Pecan stem *Phylloxera* (Homoptera: Phylloxeridae) galls as oviposition sites for overwintering generation of hickory shuckworm (Lepidoptera: Tortricidae) in native pecan groves. *J. Kans. Entomol. Soc. 6*, 428-432.

Hall, M. J. (1983): An overview of hickory shuckworm research - Has it lead to an adequate pest management program. *Entomol. Soc. Am. Misc. Pub. 13*, 47-55.

Hansen, C. D., H. W. Van Cleave and J. J. Welch (1970): Comparison of hickory shuckworm infestation rates of seven named varieties in central Texas. In: *Pecan Research 1965-1969. Tex. Ag. Exp. Stn. Prog. Rep. 2718.* 42-43.

Johnson, A. A. (1989): A study of late season hickory shuckworm activity in Southeast Kansas pecan orchards. *M. S. Thesis. Pittsburg State University, Pittsburg, Kansas USA*. 23.

McDonough, L. M., R. D. Eikenbary, M. T. Smith, M. G. Davis, C. L. Smithhisler, G. H. Hedger, R. K. Campbell, J. A. Payne, W. Reid and J. R. McVay : Sex pheromone of the hickory shuckworm *Cydia caryana*: Development of an effective field lure. *J. Chem. Ecol.* (in press)

Mitchell, P. L., R. Olszak, M. B. Stoetzel and M. K. Harris (1984): Fauna associated with galls of *Phylloxera* spp. on pecan. *Southwest Entomol. 9*, 117-124.

Moznette, G. F. (1941): Progress in the control of the hickory shuckworm on pecan. *Proc. S. E. Pecan Growers Assoc. 35*, 42-48.

Moznette, G. F., T. L. Bissel and H. S. Adair (1931): Insects of the pecan and how to combat them. *USDA Farmers Bull. No. 1654.*, 1-60.

Neel, W. W. (1959): Shuckworm control tests in Mississippi during 1957-1969 with special reference to foliage sprays and granular insecticides. *Proc. S. E. Pecan Growers Assoc. 52*, 83-87.

Osburn, M. R., W. C. Pierce, A. M. Phillips, J. R. Cole and G. L. Barnes (1963): Controlling insects and diseases of the pecan. *USDA Agric. Handbook No. 240*, 1-55.

Payne, J. A. and E. K. Heaton (1975): The hickory shuckworm: Its biology, effect upon nut quality and control. *Ann. Rep. Northern Nut Growers Assoc. 16*, 19-25.

Smith, M. T., L. M. McDonough, S. Voerman, H. G. Davis, R. D. Eikenbary and C. L. Smithhisler (1987): Hickory shuckworm *Cydia caryana*: Electroantennogram and flight tunnel studies of potential sex pheromone components. *Entomol. exp. appl. 44*, 23-30.

Sparks, D. (1977): Notes on the "Stuart" pecan. *Pecan South 4*, 204-207.

Tedders, W. L. and G. Edwards (1970): Activity of hickory shuckworm in traps. *J. Econ. Entomol. 63*, 1610-1611.

Walker, F. W. (1933): The pecan shuckworm. *Florida Agr. Exp. Stn. Bull. No. 258.*, 1-18.

Welch, J. J. and H. W. Van Cleave (1967): Initial studies on the biology and control of the hickory shuckworm in Texas. *Texas Pecan Growers Assoc. 46*, 52-56.

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STYLET PENETRATION PARAMETERS FROM APHIDS IN RELATION TO HOST-PLANT RESISTANCE.

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ABSTRACT

Electrical penetration graphs (EPGs) show the recorded activities during plant penetration by aphid stylets. Mechanical work by the stylets, punctures of plant cells, saliva secretion, active and passive ingestion and other features can be derived from these graphs. The techniques have been used in studies on host-plant resistance in order to infer the mechanism(s) of resistance. However, these studies could not explain the underlying mechanism much more than was already known from other types of experiments. Nevertheless, a number of parameters derived from these techniques was closely correlated with either susceptibility or resistance in plants to aphids. This paper aims to review these correlations and to discuss the shown potency of data from electrical penetration graphs (EPGs). Results from recent studies on resistant and susceptible lettuce lines will be used to support prospects to reveal mechanism features.

Key words: aphid, stylets, cell puncture, electrical recording, phloem sap, sieve tube, plant penetration, phloem ingestion, ultrastructure.

INTRODUCTION

Stylet penetration activities during plant penetration by aphids can be visualized by using the electrical penetration graph (EPG) technique or its precursors. Mechanical work by the stylets, the puncture of plant cells, the secretion of saliva, active and passive ingestion and other features can be derived from these graphs as summarized by Tjallingii (1988) and earlier by Tarn and Adams (1982). Eight distinct EPG wave-forms have been described, labelled mainly alphabetically A to G. The electrical recording techniques have been used in studies on host-plant resistance in order to study the process(es) of resistance. Some parameters derived from these techniques have been shown to be closely correlated with either susceptibility or resistance of plants to aphids (e.g. Kennedy et al., 1978; McLean and Kinsey, 1968; Montllor and Tjallingii, 1989; Montllor et al., 1983; Nault and Styer, 1972; Nielson and Don, 1974; Ryan et al., 1987). Although the parameters used by various authors are not always strictly comparable, some are so similar that it is possible to compile a summary of their relations to resistance of plants (Table 1).

Although these relations are rather consistent, such studies have done little more to explain the underlying causes of resistance than was already known from other

Table 1 Parameters used, and how they correlate with resistance (+) of plants, with susceptibility (-), or with neither (0).

parameter	correlation with resistance
1. Number of probes	+
2. Duration of probes	-
3. Duration of non-probing	+
4. Duration of pathway activity	0/+
5. Duration of sieve element ingestion	-
Time needed to reach sieve elements:	
6. - from start of assay	+
7. - from start of penetration	0
8. Mean duration of 'naturally' ended sieve element ingestion periods	0

types of experiments, i.e. confirming of known plant resistance or susceptibility or a rough indication at most of the tissue where a possible factor might be located. Clearly there is room for an improved use of already recognized parameters or others that can be derived from them, e.g. by better standardization of pretreatments, assay time, etc. combined with better definitions and descriptions of the wave-forms themselves; but development of new parameters through a new and more detailed knowledge of aphid-plant interactions, may allow more precise conclusions on resistance mechanisms.

POSSIBLE NEW PARAMETERS

The intracellular punctures which can be distinguished in EPGs have not yet been used to interpret specificities in the interaction of aphid and host-plant. The intracellular position of the stylet tips is reflected by a low voltage level of the recorded signal, occurring in several EPG patterns. The low voltage is due to the transmembrane potential (Tjallingii, 1985) of the punctured cell. When the stylet tips pierce the membrane, the -20 to -180 mV potential in the protoplast is electrically connected via the stylet canals, the aphid body, and the dorsal electrode to the amplifier with a high input resistance. Fig. 1 includes examples of three of the five known patterns (excluding 2 and 5) listed below. During some of these patterns, the stylet tips could be located in different tissue positions; during others, the positions are not yet known:

pattern	tissue position
1. potential drops (pds)	all living cells
2. repetitive pds	unknown
3. intracellular E1	unknown
4. E2<15 min*	unknown
5. E2>15 min	sieve elements

(* earlier an 8 min criterion was used)

Here we will discuss the properties and occurrence of these intracellular patterns and their possible relevance to the process of selection of a food plant, a factor which has been shown to be significant in some types of host plant resistance.

POTENTIAL DROPS

The first type of puncture has been described earlier (Tjallingii, 1985, 1988) as a sudden drop of the signal potential during 5 - 15 s with, during which three successive wave-form phases I II and III are displayed, and a fast return to the original level. In *Aphis gossypii* and in *Myzus persicae* it was found (Tjallingii and Romanow, unpublished results) that 50-60 % of individuals (n=26 per species) showed a pd within the first 30 s of the initial probe on two muskmelon cultivars, one susceptible and the other resistant. It seems likely that the stylets had punctured no deeper than the level of epidermal cells. In a different experiment with *Brevicoryne brassicae* on rape

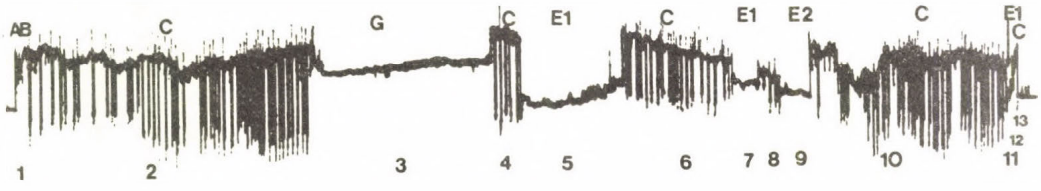

Fig. 1 EPG of a 4 hour stylet penetration by *Aphis fabae*. Explanation of the successive phases and wave-form patterns:
(1) brief period of patterns A and B representing cuticle penetration and detectable sheath secretion respectively.
(2) about one hour of pattern C, general pathway activities, including 55 potential drops (pd) each indicating a brief intracellular stylet puncture.
(3) 40 min pattern G, indicative for xylem ingestion.
(4) 7 min pathway activity including 5 intracellular punctures.
(5) 23 min of E1 on intracellular (low) voltage level, indicating an intracellular puncture. It is unknown however, what type of cell or activity is involved.
(6) 25 min pathway activity with 24 intracellular punctures.
(7) 6 min of intracellular E1 again (see (5)).
(8) 5 min pathway activity with 3 intracellular punctures.
(9) 1.5 min E1 followed by 5.5 min of E2, which is only indicative for sieve element ingestion when maintained for more than 15 min. Therefore it might be related to a sieve element puncture, but this is not sure, and anyhow the cell puncture is stopped, and so the cell is left.
(10) 45 min pathway activity with 41 intracellular punctures.
(11) 1.5 min of intracellular E1 (see (5)).
(12) 1 min of pathway without intracellular punctures.
(13) END of penetration.

seed (Fig. 2), the intervals between pds consistently decreased from the beginning of a probe towards a sieve element puncture with ingestion (E2>8 min).

The EPG in Fig. 1 shows of a complete penetration by *Aphis fabae* of a broadbean plant with numerous cell punctures during pathway activity, i.e. pattern C. The number of pds exceeds the number of cells hence suggesting multiple punctures of each cell. The fine structure of a similar penetration by the same individual, serially sectioned for transmission electron microscopy (TEM, Hogen Esch and Tjallingii, poster in this symposium), showed cells with morphological evidence of such multiple punctures, although the puncturing of a few mesophyll cells may be ambiguous. Micrographs show salivary sheath material inside breaks the walls of cells but not in the protoplasts (Fig. 3). This is consistent with earlier TEM work which showed that punctured sieve elements (Kimmins and Tjallingii, 1986) and xylem elements (Spiller and Tjallingii, in press) do not contain any sheath material. Presumably, also similar to sieve element punctures, only the maxillary stylets are inserted during the punctures of other cells.

Although most probes show abundant pds, some seem devoid of them. These are mostly brief probes, whereas the preceding and subsequent, somewhat longer probes may have normal numbers of pds. We also found that *M.persicae* may show periods of pathway activity without the usual pds (C-pd, Montllor and Tjallingii, 1989), typically on a resistant line of lettuce. The susceptible line in this study did not show

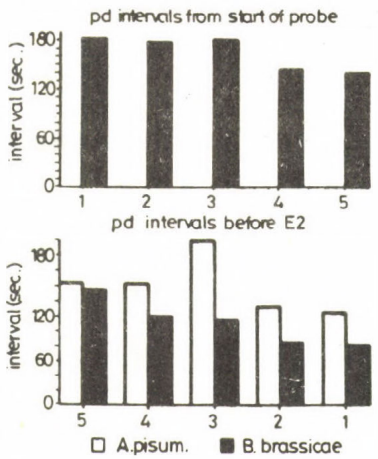

Fig. 2 Decreasing intervals between potential drops. Mean intervals plotted in 5 consecutive periods of 5 min from the start of a probe (top) by *Brevicoryne brassicae*, and preceding a period of sieve element ingestion (bottom, E2>8 min) by *Acyrthosiphon pisum* on broadbean and *B. brassicae* on rape seed (n=10).

such (C-pd) periods, neither did *Nasonovia ribisnigri* which was also tested in this experiment.

Since the pd reflects the transmembrane potential of the punctured cell, the penetrating aphid can be used as a micro-electrode measuring the membrane potentials of cells along the stylet pathway. Plotting these values in a graph provides a profile or gradient of membrane potentials from epidermis to the sieve tubes.
Fig. 4 shows such profiles for *A.fabae* on broadbean. Profiles of simultaneous probes by different individuals on the same leaf, or of consecutive penetrations by one individual in different parts of one leaf, or in different leaves demonstrate that membrane potentials may vary considerably. Profiles from the same leaf were no better related than those between leaves, between plants, or between aphid species. Fig. 1 shows that clusters of consecutive pds may be rather different to other clusters in relation to their mean magnitude, i.e. the visible length of the vertical downward deflections in the graph. There appears to be a slight tendency for the magnitude of the pd of a sieve element to be a little smaller than the directly preceding pds, which might imply a small gradient between a sieve element and its directly surrounding cells. If gradients play a role in seeking sieve elements than, more likely, gradients of plant chemicals (including pH and osmotic values) may be candidates. The numer-

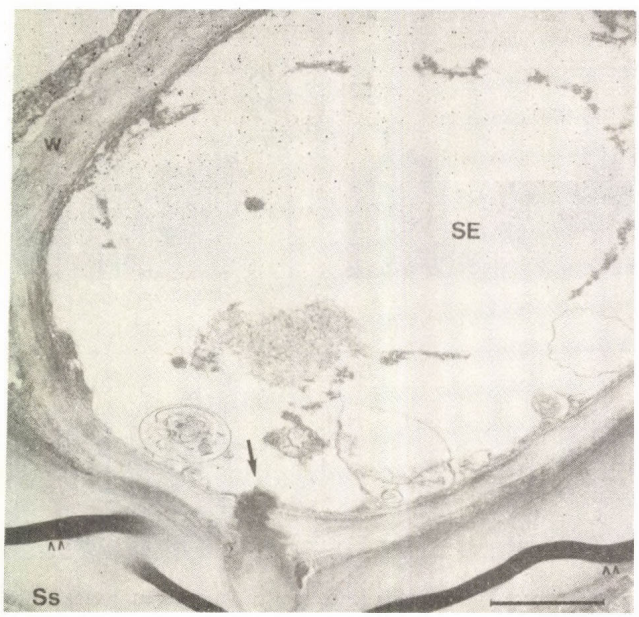

Fig. 3 Micrograph showing a small break (arrow) in the cell wall of a sieve element touching an empty branch of the stylet pathway. The salivary sheath material (Ss) has filled the break but remains outside the plasmalemma of the healthy cell. The sheath material shows some folds (^ ^) in the section, which are often unpleasant artefacts.

ous cell punctures may suggest that sieve elements are selected by testing every encountered cell.

The wave-form related to a potential drop seem to indicate in a reproducible manner a sequence of activities which is closely linked to insertion and withdrawal of the plasmalemma by the stylets. It remains a matter of speculation whether membrane penetration is a more or less automatic consequence of activities that start with extension of the maxillary stylets through the cell wall, or whether these wave-forms might be separately initiated in response to perception of the voltage change that causes the pd. It is speculative whether puncture of the tonoplast, i.e. the vacuole membrane of a cell, is an integral part of processes that together produce a pd; most plant cells, except for the companion cells and some other vascular parenchyma cells, have very little space between the two membranes so that it is likely that puncture of the tonoplast will inevitably follow. This is pertinent to the role of secondary plant substances since most of these chemicals are stored in the vacuoles and do not occur in other compartments of the plant or only in low concentrations. Also, when the tonoplast is efficiently repaired after a puncture and only a few cells are damaged, mixing of secondary products in the vacuoles with enzymes in the protoplast or cell wall (Matile, 1984) will be avoided by stylet penetration of aphids. As a consequence, the association of many of these secondary products with glucose or amino acids in their stored form, which makes them considerable less toxic, remains mainly intact. Therefore, to aphids a plant may be less toxic than to chewing insects who mix all

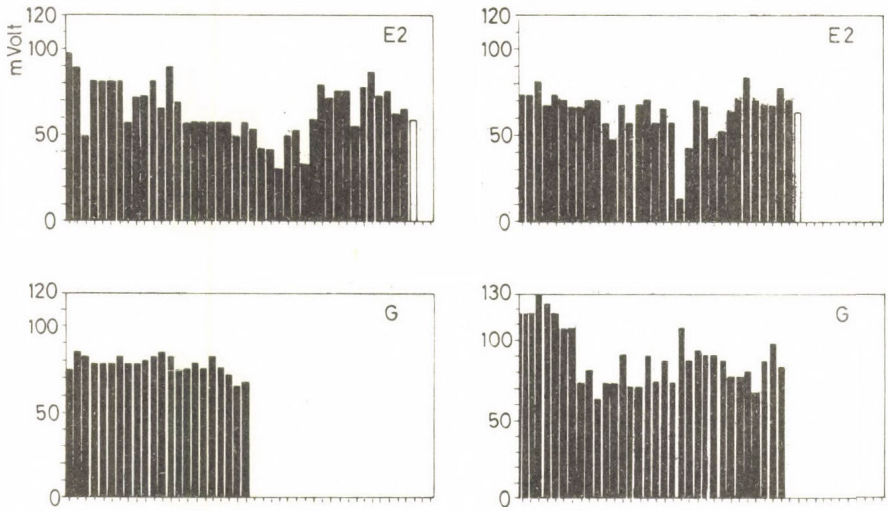

Fig. 4 Profile of subsequent membrane potentials of cell punctured during a probe en route from epidermis to the vascular bundle. Two probes end in a xylem element and produced the corresponding wave-form G, and the other two ended in a sieve element producing E2. The latter two probes end with an open bar representing the sieve element membrane potential.

the separate compartments of the plant so that the enzymes will split off the glucose and amino acid groups.

As has been distinguished earlier (Tjallingii, 1985) a regular, repetitive type of pd occurs in a number of aphid species. These pds have a longer duration, deviate with respect to phases I, II and III from normal pds, and either precede or follow periods of E2. Their features and occurrence are very similar to what has been described as X-waves by workers using the American AC recording system (McLean and Kinsey, 1968; Nault and Styer, 1972). The repetition rate of the punctures makes it difficult to imagine that more than one cell is involved, hence it is either the sieve element or any adjacent cell. The occurrence of repetitive pds is not an obligatory phenomenon. In subsequent probes by the same aphid, E2 may be preceded by the usual pds. It remains unclear why they occur in only some penetrations and by only a limited number of aphid species. The distinction between these two types of pds remains undiscussed in literature on AC-systems, presumably because the amplifiers used are not able to record them properly.

OTHER INTRACELLULAR PUNCTURES.

From the fine structure of breaks in the cell wall (Fig. 3) it cannot be inferred yet, if ever, whether an intracellular puncture really has occurred and, if so, what type of intracellular puncture in terms of wave-forms was responsible. The EPG in Fig. 1 shows that intracellular E1 and E2<15 min, both long intracellular punctures in relation to a pd, do not occur immediately at the start of a probe but later on, even after a xylem puncture, an event that most likely, but not necessarily, indicates that the stylets have reached the vascular tissue. Relating a particular vascular element to these wave-forms is difficult since the mean number of punctures for each of the vascular cell types found in the TEM study by Hogen Esch and Tjallingii (see this volume) is much higher than the number of long intracellular punctures occurring in a probe. Whether the traces left in the tissue by pds and other punctures can be distinguished needs further research. In what types of cells stylets do occur during intracellular E1 and the E2<15 min remains an open question. If they are located in sieve elements, why does the aphid not switch to E2, or why is an E2<15 min not sustained longer, which is what normally occurs during feeding punctures in sieve elements (Tjallingii, 1989)? In the TEM study of *A. fabae* on its normal host plant *Vicia faba* (broadbean), the insect finally accepted only one out of the 11 sieve elements which had been punctured all previously. Why were these sieve elements rejected? Similar phenomena seem to occur with other aphid species on susceptible food plants.

CONCLUSIONS

EPGs as well as a preliminary interpretation of TEM results suggest that cell puncturing forms a distinct behavioural unit which interrupts the other pathway activities: the wave-form phases, although not yet experimentally correlated with particular activities, seem closely linked with the recorded edges of the potential drop and the ultrastructural features suggest a delicate puncturing which differs from the normal pathway activity, where the stylets are forced between the cells and masses of sheath material are secreted. The first shape of the phenomenon of the intracellular puncture becomes visible but more experimental, morphological, and especially, chemical evidence of what happens must still be provided.

Among the parameters identified from electrical recording techniques, intracellular punctures have not yet been used to interpret host plant resistance, or food plant selection in general. It is too early however, to decide that they play a decisive role in this selection behaviour. The punctures may lead to shorter probes on non-host plants but the aphid seems difficult to convince of the unacceptable properties since the probes are repeated frequently and sieve tubes need to be reached even for a definitive decision to rejection. Host plant selection by aphids includes more than a complex manner of acquiring sensory input.

REFERENCES

Kennedy, G. G., D. L. McLean and M. G. Kinsey (1978): Probing behaviour of *Aphis gossypii* on resistant and susceptible muskmelon. *J. Econ. Entomol. 71*, 13-16.

Kimmins, F. M. and W. F. Tjallingii (1986): Ultrastructure of sieve element penetration by aphid stylets during electrical recording. *Entomol. exp. appl. 39*, 135-143.

Matile, P. (1984): Das toxische Kompartiment der Pflanzenzelle. *Naturwiss. 71*, 18-24.

McLean, D. L. and M. G. Kinsey (1968): Probing behavior of the pea aphid, *Acyrthosiphon pisum*. II. Comparisons of salivation and ingestion in host and non-host plant leaves. *Ann. Entomol. Soc. Amer. 61*, 730-739.

Montllor, C. B., B. C. Campbell and T. E. Mittler (1983): Natural and induced differences in probing behavior of two biotypes of the greenbug, *Schizaphis graminum*, in relation to resistance in sorghum. *Entomol. exp. appl. 34*, 99-106.

Montllor, C. B. and W. F. Tjallingii (1989): Stylet penetration by two aphid species on susceptible and resistant lettuce. *Entomol. exp. appl. 52*, 103-111.

Nault, L. R. and W. E. Styer (1972): Effects of sinigrin on host selection by aphids. *Entomol. exp. appl. 15*, 423-437.

Nielson, M. W. and H. Don (1974): Probing behavior of biotypes of the spotted alfalfa aphid on resistant and susceptible alfalfa clones. *Entomol. exp. appl. 17*, 477-486.

Ryan, J. D., K. W. Dorschner, M. Girma, R. J. Johnson and R. D. Eikenbary (1987): Feeding behavior, fecundity, and honeydew production of two biotypes of greenbug (Homoptera: Aphididae) on resistant and susceptible wheat. *Environ. Entomol. 16*, 757-763.

Spiller, N. J., and W. F. Tjallingii (1989): Xylem ingestion by aphids. *Entomol. exp. appl.* (in press).

Tarn, T. R. and J. B. Adams (1982): Aphid probing and feeding, electrical monitoring, and plant breeding. In: K. F.Harris and K. Maramorosch (eds), *Pathogens, Vectors and Plant Diseases: Approaches*. Academic Press, New York.

Tjallingii, W. F. (1985): Membrane potentials as an indication for plant cell penetration by aphid stylets. *Entomol. exp. appl. 38*, 187-194.

Tjallingii, W. F. (1988): Electrical recording of stylet penetration activities. In: A. K. Minks and P. Harrewijn (eds), *Aphids, their Biology, Natural Enemies and Control*. Vol. 2B, Elsevier, Amsterdam.

Tjallingii, W. F. (1989): Continuous recording of stylet penetration activities by aphids. In: R. D. Eikenbary and R. K. Campbell (eds), *Mechanisms of Aphid-Plant Genotype Interactions*. Symp. XVIII Int. Congr. Entomol. Vancouver, 1988. Elsevier, Amsterdam. (in press)

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THE IMPORTANCE OF HOST PLANT – INSECT RELATIONS IN THE PEST MANAGEMENT PROGRAMME ON MAIZE IN AFRICA

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ABSTRACT

The basic difference existing in the oviposition behaviour, the 1st larval instar movement and the preference for feeding of older larval instars of *Chilo partellus*, *Busseola fusca*, *Sesamia calamistris* and *Eldana saccharina* have determined the methodology and the success of resistance screening and breeding of maize to stem borers. Various mechanisms involving oviposition and larval feeding behaviour of *Chilo* were identified in resistant maize and sorghum lines. The knowledge of these mechanisms should help breeders in their strategy to develop a new germplasm combining different mechanisms of resistance.

Key words: *Chilo partellus*, *Busseola fusca*, *Sesamia calamistris*, *Eldana saccharina*, *Chilo orichalcociliella*, *Sesamia botanephaga*, maize, plant resistance, sorghum.

The objective of the present paper is not a comprehensive review of existing published information widely scattered in various reports and journals, but the author's personal experience gained during a nine-year research and interactions with other scientists working on plant resistance to insect pests in East and West Africa. In spite of practical objectives to select and breed resistant cultivars suitable for different ecological zones in Africa, all subsequent techniques and methods used in these projects were a consequence of extensive fundamental observations and experiments on biology, behaviour, host plant relations and ecology of the target species.

All newly initiated projects on plant resistance in Africa have always faced some classical barriers as: (1) the lack of adequate published information on a number of basic relationships between most insect species and crop plants; (2) lack of well trained research and technical stuff, and (3) lack of sufficient facilities and infrastructure for basic research (Dabrowski, 1980). These barriers in developing fundamental research in entomology have been already obvious for some time to scientific leaders in Africa and at least some of them have been successful in creating independent research centres. Prof. Thomas R. Odhiambo, with support of the Kenyan government, some national academies of science of developed countries and international organizations, has established the International Centre of Insect Physiology and Ecology in Kenya being presently the world centre of entomological research in the tropics. Already in 1975, he acknowledged the importance of fundamental research in developing new strategies in pest control in Africa by stating

that the chemical control of insects in the tropics "... has seen some spectacular achievements, especially in the short-term. But they also do not seem to offer a long-lasting, environmentally acceptable solution... We should cast our minds for a more scientific method, which recognizes that insects are not simply targets, but are biological entities with a complex ecology, behaviour, and physiology. Such an approach, a pest management approach, now seems to be emerging from the obscure horizons. It presupposes a great deal of fundamental information on the individual insect species... if we are to suggest new avenues for controlling insect pest species within acceptable population limits" (Odhiambo, 1975).

In most of the present research project conducted by national agricultural institutes and by international teams supported by FAO, UNDP, ICIPE, IITA, CIMMYT, WARDA and ICRISAT in Africa, the focus is on developing non-chemical strategies in pest control based on cultural and biological methods and on the introduction of resistant and improved cultivars. The use of resistant cultivars as a method of crop protection has gained acceptance in tropical countries. Host plant resistance is now considered to be one of the primary lines of defence in all pest management programmes for small farmers. Introduction of new resistant cultivar releases farmers from worrying about technological aspects, such as timing of applications, dosage of chemicals or biological agents. There is also no direct cost to growers. The latter two considerations make the use of resistant plants important to both developed and developing countries and they should thus form an integrated part of a pest management programme.

It must be stressed, however, that while plant resistance to insects is a highly promising strategy of pest control, it requires sustained long range work and a joint action of research by entomologists, agronomists, plant breeders and geneticists, plant and insect physiologists and chemists. These factors, as well as financial and logistic constraints are often hampering progress in resistance breeding programmes in Africa.

SPECIES COMPOSITION OF STEM BORERS ON MAIZE

Stem borers are considered major pests of graminaceous crops worldwide. In the temperate regions, relatively few species of important stem borers are known. In the tropics, these pest species are more numerous and reduce considerably the yield of maize, and the other crops most widely grown in the developing countries. It is estimated that the stem borers are responsible for 15-78% (but often 100%) of yield losses in maize in Africa. Stem borers attacking maize and sorghum in the tropics caused "dead hearts" in young plants, in older plants the upper part of the stem usually dies due to the boring of the larva in the stem pith. The cobs are also attacked.

There are six major stem borer species attacking maize in Africa: *Busseola fusca* (Fuller), *Sesamia calamistris* Hamps., *S. botanephaga* Tams and Bowden, *Eldana saccharina* Wlk., *Chilo partellus* (Swinhoe) and *Chilo orichalcociliella* (Strand). *C. partellus* has become a maize pest in Eastern and Southern Africa only in recent

times. It is thought to have invaded the continent from Western Asia and is gradually becoming more important than *C. orichalcociliella*, which is native to East Africa. In East Africa, *B. fusca* occurs in the cooler high lying inland areas, while *S. calamistis* occurs in the warmer coastal regions. In West Africa, *S. calamistis* occurs in the humid lowland areas and *B. fusca* in the savannah zone (Harris, 1962; Usua, 1968). In some areas of the lowland rain forest *S. botanephaga* became a dominant species, often confused with *S. calamistis* (Harris, 1962; Endrődy-Younga, 1968). The first recorded outbreak of *E. saccharina* in Africa was observed on sugarcane in Tanzania in 1959 and in Uganda in 1967. Since that time it has been an important pest of maize in most countries (Girling, 1978). *Eldana* larvae first appear shortly before the host plant becomes to bloom. The number increases steadily toward the period of cob ripening and reaches its peak after the harvest, if the plant is allowed to remain standing in the field. It is a dry-season oriented species, the population is generally larger during that period.

CHILO PARTELLUS

A project on maize resistance to stem borers has been initiated in 1978 at ICIPE with the research work being concentrated at the Mbita Point Field Station at the shores of Lake Victoria on the following aspects: (1) field screening of recent and old local cultivars and lines originating from national and international plant breeding institutes (IITA, CIMMYT, ICRISAT). The screening has required an estimation of reliable and efficient screening techniques (Dabrowski and Omolo, 1980). Where artificial infestation was necessary, procedures for mass rearing of the insect species tested were elaborated. Screening under heavy natural infestation was always compared to the artificial infestation done under screenhouse and field conditions. (2) Studies on mechanisms of plant resistance including all aspects of behavioural and physiological relationships between insect pest and crop plant tested. The investigations on mechanisms involved in oviposition behaviour of adult insects and larval feeding behaviour were initiated not only for the purpose of studies on plant resistance, but also to find the best technique for insect mass rearing. Without a detailed information on plant properties affecting the insect in its search, recognition and acceptance of a plant as food, it would be difficult to choose the optimal artificial surface for oviposition or to compose artificial diets for larvae.

Initial screening of one hundred different maize cultivars and inbred lines from national programmes in East Africa under heavy natural infestations of *Chilo* showed the existence of resistance in some local cultivars (Omolo, 1980; 1983). The inbred A used widely in Kenyan National Maize Breeding Programme for hybrid production showed high level of susceptibility. The line was developed by breeders in the 70's under chemical control of pest protection and it had the best combining ability with other inbred lines. Inbred A is also high yielding when protected against stem borers. Two other inbred lines, D and G, were identified as resistant. Preliminary observations on the females' oviposition behaviour suggested that antixenosis for oviposition

was an important type of resistance. The preference for oviposition was measured by the number of eggs laid by *Chilo* females released in screenhouses with maize line planted in choice and non-choice arrangement. The maize lines which were hairy on the upper part of the leaf (inbred D, G) were less oviposited, but were as much oviposited as the others on the lower surface of the leaf, which was glabrous in all lines tested. Pilosity or some unknown factor associated with it had a negative influence on oviposition.

The choice test showed, however, much greater difference between highly susceptible inbred A and the moderately resistant inbred D and G than observed under non-choice situation (Dabrowski and Nyangiri, 1983). Because crops are rather grown in the non-choice situations where the cultivar occupies a larger area, it was decided to continue search for higher level of resistance in exotic germplasm provided by CIMMYT, Mexico.

Five hundred full and half-sib families developed by CIMMYT from three basic populations (IDRN-Cornell, Population 25 and 27 that had been improved for resistance to other species of stem borers in the USA and Mexico) were tested for *Chilo* resistance under heavy natural and artificial infestation. The resistance identified in some lines was higher and more broadly based than the local source (Dabrowski et al., 1981; Omolo, 1983). This success in resistance screening in this rather short period was mainly possible due to the variability existing in world maize germplasm and specific oviposition and feeding behaviour of *Chilo*. The newly hatched larvae either climb the plant to the whorl where they feed and may become established or they leave the plant before the whorl is reached. Resistance to stem borers may result simply from failure of the larvae to reach the feeding site (Dabrowski et al., 1981; ICIPE, 1982). The results of all experiments on maize resistance to *Chilo* suggest that there are at least five levels of relationships affecting plant colonization, larval survival and damage level: (1) non-acceptance for oviposition: inbred D and G, CIMMYT line 125, 33, 324, 178; preference to the inbred A (2-3 times more eggs oviposited); (2) feeding of young larval instar on young leaves and leaf sheats: reduced on CIMMYT line 324, 125, 22; (3) concentration of feeding of young larval instars on the upper growing part of plant: extensive on the highly susceptible inbred A and reduced on all resistant lines tested; (4) penetration of young larval instars into stem: reduced on CIMMYT line 341, 125 and inbred G; (5) feeding of older larval instars in stem: reduced on CIMMYT line 342, 125, 28 and inbred D (Dabrowski and Nyangiri, 1983). Later experiments of Ampofo (1985, 1986) confirmed these relations for other sources of resistance, especially from lines developed in Mississippi, USA.

BUSSEOLA FUSCA

A moderate level of resistance in maize was identified for another species of stem borers – *Busseola fusca*, whose oviposition and larval behaviour is similar to *Sesamia* (Barrow, 1985). The differences in the extent of leaf damage, larval survival and

development, was attributed by Barrow (1985) to the presence of either one or two resistance factors: a short-lived, but effective factor reducing larval numbers and a second, longer lasting factor retarding larval development.

The resistance screening of maize to *Busseola* is hampered by the lack of a successful method of mass rearing on artificial diet caused by occurrence of high portion of diapausing larvae in the 2nd and 3rd generation kept under insectary conditions (Dabrowski, 1984).

SESAMIA CALAMISTIS AND *ELDANA SACCHARINA*

The progress in resistance screening of maize to *Sesamia* and *Eldana* is less spectacular, probably due to differences in the type of relations between these species and the maize plant. *Sesamia* egg masses are deposited between leaf sheath and stem and young larvae tunnel directly into the stems soon after hatching, although some may feed on the leaf whorl and sometimes on upper leaves. They also attack cobs.

To facilitate screening maize germplasm for resistance to *Sesamia* under uniform infestation, large scale rearing procedure on artificial diet was developed at IITA in 1982 allowing production of 8-10 thousand insects weekly (Dabrowski and Jackai, 1983). Some levels of resistance in maize germplasm was identified under screenhouse conditions, when infestation was done on 4-7 leaf stages of maize (Dabrowsky, 1983), but it was difficult to produce similar reproducible differences under field conditions either under natural or artificial infestation (Mareck et al., 1985; IITA, 1987a). In the second growing season, the maize crop is under continuous pressure from *Sesamia* population migrating probably from drying grasses, therefore, resistant plants should possess such mechanisms of resistance that would act in subsequent growth stages of plants and on various *Sesamia*/host plant relations. Resistance screening under artificial infestation to *Eldana* was only recently initiated at IITA, Nigeria, after developing mass rearing techniques on artificial diets (Dabrowski and Jackai, 1983; IITA, 1987b) and conducting detailed observations on *Eldena* oviposition and larval feeding behaviour. Seedlings or young plants of preflower stage are seldom attacked, although when reared on these materials in the laboratory, larvae developed normally. They also bore down into the root stock – but at heavy infestations they can be found in any internode along the stem, and they often bore through the nodes into other joints. The oviposition preference was observed to dry leaves or dry trashes, therefore, tissue paper was used as an artificial surface in oviposition cages.

For artificial infestation, egg masses were placed between the leaf sheaths at the level of the ear node or above the ear. Less larval establishment was observed when the egg masses were placed close to the base of the plants (IITA, 1987a). Significant differences in the percent of nodes bored by *Eldana* larvae on some maize plants were found at IITA (IITA, 1987a), where the resistance screening and breeding is continued.

CONCLUSIONS

The basic information on stem borers/maize plant relations are fundamental to develop a new strategy in pest management of maize crop (Fig. 1). Our knowledge on larval behaviour of stem borers may explain successful chemical control of *Chilo* and *Busseola* problems in the control of *Sesamia* and *Eldana*, either by synthetic insecticides, as well as natural products (Dabrowski et al., 1984; Dreyer and Dabrowsky, unpublished). The efficiency of some insecticides and various extracts from neem seed powder rapidly decrease with the increase of elapsed time between hatching of *Sesamia* larvae and application. The high mortality of young larvae was noticed up to the 5th day and rapidly reduced by the 10th and 15th day after infestation, when most of the larvae penetrated the stem and escaped the insecticides.

The restrictive use of synthetic insecticides as seed dressing in the pest management programme on maize in Africa should be advocated. Maize growing area is on the increase in all climatic zones, as the farmers' response to the public demand for some food crop for rapidly increasing human population. The technology of production is changing from traditional shifting cropping to the more intensive. The introduction of zero-tillage reducing soil erosion caused the increase of damage of maize seedlings by coleopterous soil pests in some areas. In the Savannah zones in Burkina Fasso (Suh et al., 1985), Nigeria (Z.T. Dabrowski, unpublished) and Cameroon (L.T. Empig, unpublished) termites, which normally feed on dried plant parts and trashes in the soil, were attacking healthy, fresh parts of maize plants, especially the base of

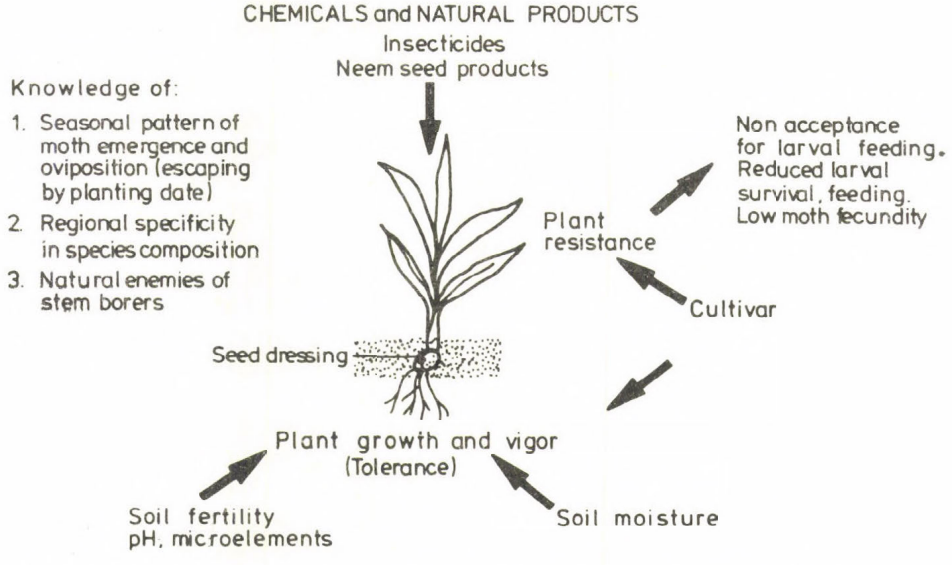

Fig. 1 Basis of integrated approach to the control of stem borers in maize in the tropics.

the plant. The change in feeding behaviour of some termite species caused already serious damage and lodging on large areas in West Africa. The status of maize pests in Africa represents a dynamic relationship between various insect species, maize plants, environment and cropping technology. The change in one component affects all other groups. The introduction of some new high yielding cultivars have increased the economic importance of some species (e.g. stored product pests). At the same time, the high level of resistance was also identified in new introductions. S.K. Kim from IITA selected a number of hybrids showing high level of resistance to *Sitophilus* sp. (IITA, 1987d). The highly yielding inbred A used widely for the hybrid production in Kenya showed the highest susceptibility to *Chilo* sp. Traditional tall maize cultivars with strong stem producing low but stable yield showed some level of tolerance to stem borers in West Africa, whereas some improved varieties with weaker stems were seriously damaged. On the other hand, the only high level of resistance to *Chilo* was identified in the exotic germplasm.

The above information showed that recent development of strategies in pest control on maize in Africa was possible because more attention was given to the studies on basic relationships between insect species, host plants, environmental factors and cropping systems. Creation of new research groups with strong training component (e.g. at ICIPE, Kenya) may garantee the transfer of this approach to IPM programmes on other crops in various national research institutions in the tropics.

REFERENCES

Ampofo, J. K. O. (1985): *Chilo partellus* (Swinhoe) oviposition on susceptible and resistant maize genotypes. *Insect Sci. Applic. 6*, 323-330.

Ampofo, J. K. O. (1986): Effect of resistant maize cultivars on larval dispersal and establishment of *Chilo partellus* (Lepidoptera: Pyralidae). *Insect Sci. Applic. 7*, 103-106.

Barrow, M. R. (1985): The effect of different maize genotypes on the maize stalk-borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), feeding in whorl tissue. *J. Entomol. Soc. South Africa, 48*, 113-119.

Dabrowski, Z. T. (1980): Host plant insect relationships and studies on plant resistance in the ICIPE in 1979. *Seventh Annual Report for 1979. ICIPE.* Nairobi, Kenya. 15-18.

Dabrowski, Z. T. (1983): Screening maize for *Sesamia* resistance. *Annual Report for 1982. International Institute of Tropical Agriculture.* Ibadan, Nigeria. 31.

Dabrowski, Z. T. (1984): Mass rearing of stem borers. *Annual Report for 1982. IITA.* Ibadan, Nigeria. 39.

Dabrowski, Z. T. and L. Jackai (1983): Rearing stem borers on artificial diets. *Annual Report for 1982. IITA.* Ibadan, Nigeria. 30.

Dabrowski, Z. T. and E. Omolo (1980): Estimation of methodology of maize screening to *Chilo partellus* infestation. *Seventh Annual Report for 1979. ICIPE,* Nairobi, Kenya. 21-22.

Dabrowski, Z. T. and E. O. Nyangiri (1983): Some field and screenhouse experiments on maize resistance to *Chilo partellus* under Western Kenya conditions. *Insect Sci. Applic. 4*, 109-118.

Dabrowski, Z. T., J. Omoreghe and E. O. Osisanya (1981): Resistance of maize to stem borers. *Eight Annual Report for 1980. ICIPE,* Nairobi, Kenya. 5-7.

Dabrowski, Z. T., J. Omereghe and E. O. Osisanya (1984): Effect of maize growth stage on *Sesamia* damage. Annual Report for 1983. IITA. Ibadan, Nigeria: 39-40.

Endrődy-Younga, S. (1968): The stem borer *Sesamia botanephaga* Tams and Bowden (Lep., Noctuidae) and the maize crop in central Ashanti, Ghana. *Ghana J. agric. Sci. 1*, 103-131.

Girling, D. J. (1978): The distribution and biology of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) and its relationship with other stem borers in Uganda. *Bull. Entomol. Res. 68*, 471-488.

Harris, K. M. (1962): Lepidopterous stem borers of cereals in Nigeria. *Bull. Entomol. Res. 53*, 139-171.

ICIPE (1982): Behaviour and survival of *Chilo partellus* on resistant maize lines. *Ninth Annual Report for 1981. ICIPE,* Nairobi, Kenya. 16-17.

IITA (1987a): Stem borer resistance screening. IITA Maize Research Program. *Annual Report 1986. IITA,* Ibadan, Nigeria. 65-68.

IITA (1987b): Rearing of stem borers. IITA Maize Research Program. *Annual Report 1986. IITA,* Ibadan, Nigeria. 62-63.

IITA (1987c): Artificial stem borer infestation. IITA Maize Research Program. *Annual Report 1986. IITA,* Ibadan, Nigeria. 63-64.

IITA (1987d): Screening for resistance to storage pests. IITA Maize Research Program. *Annual Report 1986. IITA,* Ibadan, Nigeria. 68-69.

Mareck, J. H., Z. T. Dabrowski, A. Carter and K. Nwosu (1985): Stem borer research. *Annual Report for 1984. IITA,* Ibadan, Nigeria. 46-48.

Odhiambo, T. R. (1975): *This is a dudu world. The Status of Insect Science in the Tropical World.* ICIPE, Nairobi, Kenya.

Omoio, E. O. (1980): Screening maize and sorghum cultivars for resistance to stem-borers complex. *Seventh Annual Report for 1979. ICIPE,* Nairobi, Kenya. 19-20.

Omolo, E. O. (1983): Screening of local and exotic maize lines for stem-borer resistance with special reference to *Chilo partellus. Insect Sci. Applic. 4*, 105-108.

Suh, J. b., M. S. Roddriguez and A. O. Diallo (1985): Screening maize for resistance to termites. *Annual Report for 1984. IITA,* Ibadan, Nigeria. 60-62.

Usua, E. T. (1968): The biology and ecology of *Busseola fusca* and *Sesamia* species in South-Western Nigeria. I. Distribution and population studies. *J. econ. Entomol., 61*, 830-833.

SUMMARIES OF POSTER PRESENTATIONS

Symp. Biol. Hung. 39, 1990

CITRUS LIMONOIDS AS INSECT ANTIFEEDANTS: DETERRENTS OR TOXINS?

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Key words: *Leptinotarsa decemlineata*, *Spodoptera frugiperda*, limonin, epilimonol, structure-activity relationship, chemoreceptor, buccal cavity, oviposition, ablation, starvation.

Limonoids are a class of modified triterpenes found in the plant families Meliaceae, Cneoraceae, and Rutaceae which have elicited antifeedant responses in many insect species. Limonin is of particular interest because it is easily isolable and purified in quantity from seed available as a by-product of the citrus industry. Also, limonin possesses a number of polar functional groups which allow for molecular modifications to examine structure-activity relationships.

We initially evaluated limonin for its effectiveness in reducing feeding of Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), and it reduced short-term (8 hr) feeding by fourth instars >60% at dosage ±10 $\mu g/cm^2$ (Alford et al., 1987). Further testing of ten derivatives indicated the epoxide function on the D-ring and the furan ring were essential for limonin's activity (Bentley et al., 1988). A synthetic model of limonin, based on the C-, D-, and furan rings, elicited activity comparable to that of limonin (Rajab et al., unpublished data).

We found limonin to be more active in no-choice assays with CPB and in choice arenas with fall armyworm (FAW) larvae, *Spodoptera frugiperda* (J. E. Smith). The gustatory mouthparts previously identified to regulate feeding by CPB and FAW larvae were removed, and for each ablation treatment, limonin still reduced feeding significantly. We concluded that limonin acts primarily as a toxin against CPB, exerting a post-ingestive effect to reduce feeding; for FAW, it may act as a toxin or as a deterrent with a site of action an unidentified chemoreceptor in the buccal cavity (Mendel et al., unpublished data).

We examined the consequences of long term (72 hr) exposure of limonin and several derivatives to CPB larval growth, development, and survival. At 32 $\mu g/cm^2$, limonin and epilimonol significantly reduced growth, delayed development, and decreased survival. Using a starvation regime as a simulation model for antifeedant effects, the primary effect of epilimonol seems due to starvation; a similar conclusion was not possible for limonin.

Epilimonol was evaluated for its effects on adult CPB feeding and oviposition. It reduced feeding >40% at dosages as low as 10 $\mu g/cm^2$, and adults did not select

untreated potato foliage over epilimonol-treated foliage (Liu et al., 1989a,b). In long term tests, epilimonol suppressed oviposition completely; starvation for 16 hr/day elicited similar responses.

REFERENCES

Alford, A. R., J. A. Cullen, R. H. Storch and M. D. Bentley (1987): Antifeedant activity of limonin against Colorado potato beetle. *J. econ. Entomol. 80*, 575-578.

Bentley, M., M. Rajab, R. Alford, M. Mendel and A. Hassanali (1988): Structure-activity studies of modified citrus limonoids as antifeedants for Colorado potato beetle larvae, *Leptinotarsa decemlineata*. *Entomol. exp. appl. 49*, 189-193.

Liu, Y.-B., A. R. Alford, M. S. Rajab and M. D. Bentley (1989a): Effects and modes of action of citrus limonoids against *Leptinotarsa decemlineata*. *Physiol. Entomol. 14*, (in press.)

Liu, Y.-B., A. R. Alford and M. D. Bentley (1989b): Effects of epilimonol and starvation on feeding and oviposition by *Leptinotarsa decemlineata*. *Entomol. exp. appl. 52*, 39-44.

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ROLE OF NONPROTEIN AMINO ACIDS IN PHAGOSTIMULATION AND SURVIVAL OF THE PEA APHID, *ACYRTHOSIPHON PISUM*

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Key words: *Acyrthosiphon pisum*, pea aphid, allelochemicals, isoxazolinone compounds.

Nonprotein amino acids are secondary plant metabolites, highly diversified, and usually occurring in the free form. In general these higher plant allelochemicals are toxic and show deleterious effects on developmental stages, fecundity and fertility of many insects and vertebrates. The present studies were undertaken with a view to assess the role of certain nonprotein amino acids occurring in *Lathyrus* or *Pisum* spp., on the pea aphid, *Acyrthosiphon pisum*.

The nonprotein amino acids L-homoarginine, lathyrine, β-(isoxazolin-5-one-2-yl)-alanine (I), isowillardiine (II), and 2-(3-amino-3-carboxy-propyl)-alanine (VI), and the metabolically related nitriles 2-cyanoethyl-isoxazolin-5-one (VIII) and β-amino-propionitrile fumarate (BAPN) where individually incorporated at 0.01, 0.05 and 0.1% in a 20% sucrose solution, at pH 7.0; other experimental details are described by Srivastava et al. (1988).

Our results showed that compounds I, VIII and L-homoarginine were highly phagostimulatory, lathyrine and BAPN had a deterrent effect, and VI and II were neither phagostimulatory nor inhibitory. Homoarginine, I, VI and VIII were beneficial for survival, whereas II and lathyrine had no effect on survival. It is suggested that *A. pisum* may have developed a metabolic process that either detoxifies or eliminates some of these nonprotein amino acids, which are generally toxic to vertebrates; the aphids may actually be using these compounds in differentiating host-plants.

REFERENCE

Srivastava, P. N., F. Lambein and J. L. Auclair (1988): Nonprotein amino acid-aphid interaction: Phagostimulatory effects and survival of the pea aphid, *Acyrthosiphon pisum*. *Entomol. exp. appl. 48*, 109-115.

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POPULATION DYNAMICS OF A LEAF-MINING INSECT AT ENDEMIC AND EPIDEMIC DENSITIES

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Key words: density dependence, larval interference, larval mortality, leaf miner, outbreak, parasitism, phenology, *Phyllonorycter*, population regulation, *Populus*.

In northern Minnesota, USA, *Phyllonorycter salicifoliella*, a leaf-mining moth, occurs on quaking aspen, *Populus tremuloides*, in a mosaic of densities ranging from endemic (<1mine/leaf) to epidemic (>5mines/leaf). Epidemic densities have persisted at some sites for at least 15 years. Since such long-lived outbreaks are rare in nature, the *Phyllonorycter*-aspen system presents an excellent opportunity to examine potential regulatory agents across a wide range of densities.

To quantify sources of egg, larval, and pupal mortality, I censused all miners on 200 marked leaves on four quaking aspen trees during the 1986 and 1987 growing seasons. I recorded density and condition of all eggs, larvae, and pupae, as well as sources of mortality for dead larvae and pupae. I also measured area of each leaf.

To test which sources of mortality operated in a density-dependent manner, I ran linear regressions of proportion of miners dying from each source of mortality (angularly transformed) vs. mine density. I ran regressions over three scales of density, per leaf, per twig, and per tree, to test whether any density-dependent effects varied with spatial scale.

During 1986 and 1987, mean densities on study trees ranged from 1.3 to 14.9 miners per leaf. Despite this variation, survivorship of miners varied little among trees or between years. Most mortality occurred during the larval stage, with parasitism, larval interference, and unknown factors being the predominant causes.

Mine density per leaf was positively correlated with leaf area. In addition, miners consistently exhibited a clumped distribution among leaves within each host tree. The only source of mortality to consistently operate in a density-dependent fashion at any spatial scale was larval interference.

Variability in leaf-miner density among trees and aggregation of miners within trees indicate that ovipositing females prefer some trees, and leaves within trees. In previous research I found the primary determinant of density to be host-tree phenology; eggs are preferentially laid on young, not yet fully-expanded leaves. The correlation between leaf size and miner density indicates that females also prefer to oviposit on larger leaves.

The clumped distribution of miners among leaves is surprising, given that larval interference is the only density-dependent source of mortality. This apparent paradox is rationalized in part by observations that winning an intraspecific encounter enhances both survivorship and future fecundity of females. The net result is that probability of emergence is independent of density on a leaf.

Much ecological theory suggests that populations of phytophagous insects are displaced from equilibrium levels by abiotic, density-independent factors and regulated by density dependent ones. This is not the case for *P. salicifoliella*. Even though larval interference is density-dependent over several spatial scales it alone is insufficient to regulate population size and reduce epidemic densities to endemic ones. Cessation of outbreaks also requires alteration of normal host-tree phenological patterns in response to atypical meteorological events.

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THE SIGNIFICANCE OF CASSAVA EXUDATE FOR PREDACEOUS MITES

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Key words: *Typhlodromalus limonicus*, cassava, exudate, bodyguard, oviposition, predation, migration, population dynamics, spider mites, thrips.

Extra-floral nectaries are present in many species of plants. The evolution of the trait is usually thought to relate to the selective advantage that plants obtain from the protection conferred by organisms foraging for extra-floral nectar. By investing in food (read nectar) for the third tropic level the plants ensure themselves the presence of 'bodyguards'. Ants are by far the most frequently observed visitors of extrafloral nectaries; all experimental work so far is exclusively related to these organisms.

We found that predatory mites (Phytoseiidae) can feed phloem exudate of cassava. Pilot experiments with 4 species of phytoseiid mites found on cassava showed that in the absence of other food sources such as spider mites and thrips, cassava exudate is an adequate alternative. On a diet exudate only, 50% of the predators were still alive after 14 days whereas in the control experiment total mortality was found after 5 days already. The diet does not allow for oviposition but egg production is resumed as soon as pray is available again. In addition to this it was found that *Typhlodromalus limonicus*, a very common phytoseiid on cassava, could also complete juvenile development on cassava exudate. Two-choice disc experiments showed that both larvae and adults of this species spend more time on sites where exudate is present. These experimental results strongly indicated a mutualistic relationship between cassava and *T. limonicus* with the following characteristics:

1) *T. limonicus* benefits from choosing cassava as a host plant because the presence of exudate enables the predator to bridge preyless periods.

2) Cassava benefits from the presence of *T. limonicus* because for its reproduction this predator entirely depends on the comsumption of organisms harmful for cassava.

The striking feature of this hypothesis is that it assumes the predators will persist on the crop in the absence of prey. Under these circumstances phytoseiids usually disperse from the hostplants. Being wingless their (passive) migration by the use of air currents is a risky venture. Hence, it is to be expected that the predators will not disperse as long as the probability to survive until the next pest infestation is higher than the probability to find a new prey patch by dispersing.

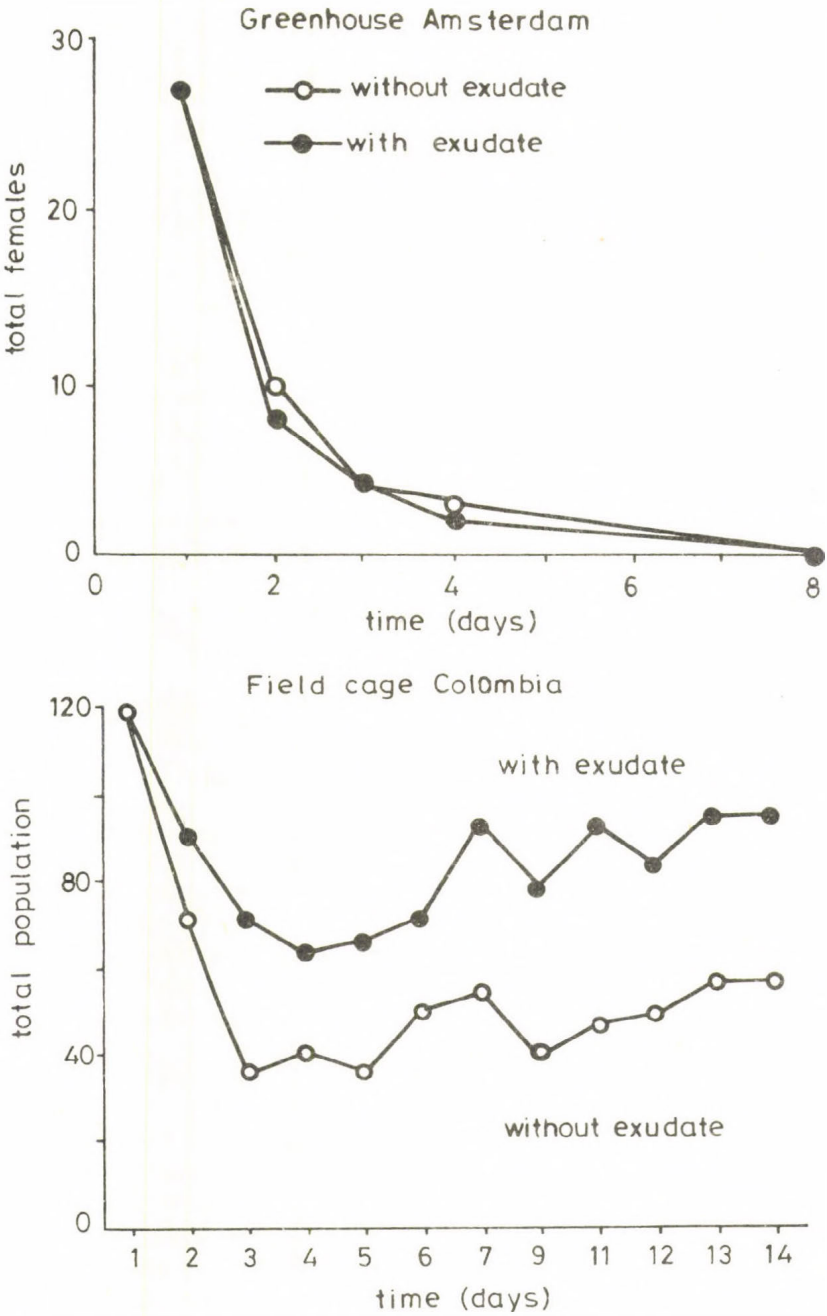

Fig. 1+2 Population dynamics of predatory mites on plants with/without exudate

The hypothesis that exudate producing plants maintain their bodyguards was tested experimentally. This was done by sealing the production site of exudate (the petiole) of 20 clean cassava plants bearing 6 leaves each with PVC-tubeletts and parafilm. Subsequently exudate droplets (3 per petiole) were applied on 10 plants. Then 12 gravid *T. limonicus* females were introduced on each plant. The population dynamics of the predators was monitored during two weeks. The experiment was done on two different location, in a greenhouse in Amsterdam and in a field cage in Colombia. The results are given in Fig. 1 and 2.

From the experiment done in Colombia it is obvious that in the presence of exudate twice as many predators persist on the plants than in its absence. It should be noted, however, that even on the plant without exudate a population of predators pesisted and some reproduction and juvenile development occurred. These plants were free of prey material. It is as yet unclear on what the predators might have fed. On the other hand, the experiments carried out in Amsterdam yielded contradictory results in all respects. There was no effect of exudate, the predators disappeared from the plants within a week, and reproduction did not occur. The only differences between the experiments were environmental, the same clone of cassava and the same strain of *T. limonicus* were used. Moreover, in a bioassay run simultaneously in the lab the exudate proved to greatly increase survival of these mites. It should be mentioned here that rearing *T. limonicus* is very difficult in Amsterdam, whereas this is not so much of a problem in Colombia. At present we are investigating what role phyllosphere microorganisms may play in the arrestment of this predator.

It can be concluded that if the environment is favourable for persistence of *T. limonicus* the production of exudate may be of adaptive significance for the plants producing it. This is because in this way they maintain a higher number of bodyguards than would be the case without exudate.

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WOUND-INDUCED CHANGES IN BIRCH AND CATERPILLAR BEHAVIOUR

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Key words: *Betula pubescens*, *Betula pendula*, *Orthosia gothica*, movement, feeding, foraging behaviour, leaf damage, wound-induced changes.

Previous studies have found that wound-induced changes in birch (*Betula pubescens* and *B. pendula*) adversely alter the growth rate and survival of larvae feeding on leaves adjacent to the damaged one (Haukioja and Niemelä, 1976) and can reduce palatability of damaged and adjacent leaves (see refs in Edwards et al., 1988). These changes create a heterogeneous environment for foraging insect herbivores which will be likely to affect their feeding behaviour (Schultz, 1983). This work investigates the behavioural basis of the highly-dispersed grazing patterns seen on previously grazed leaves (Silkstone, 1987). It follows previous work (Edwards et al., 1988) showing increased movement and decreased grazing levels on damaged compared with control foliage.

Questions asked: (1) How does damage to birch foliage affect caterpillar foraging behaviour? (2) How does damage affect the distribution and amount of subsequent insect feeding?

The experiments used mature birch trees (*B. pendula*) from which twigs with six undamaged leaves either had three alternate leaves damaged or were left as controls. After a further period on the tree, the twigs were cut and placed in water. Two caterpillars of the Hebrew character moth (*Orthosia gothica*, Lepidoptera: Noctuidae) were placed on each and their behaviour recorded every half hour. There were 2 sets of treatments: Experiments (1) and (2): control; artificially damaged leaves (ca. 20% of area removed); caterpillar damaged leaves. Experiments (3) and (4): control; light artificial damage (ca. 10% removed); heavy artificial damage (ca. 30% removed).

(1) Effects of damage on behaviour

a) Effects on movement: Observing the numbers of movements per caterpillar per hour (where 1 movement is a standard distance travelled) showed that movement was infrequent but more common on the controls.

b) Effects on feeding: The number of animals feeding at each half-hour interval was recorded. These are often similar, although in Exp. (3) more meals were recorded on the damaged leaves. However, this is a very crude way of estimating the true number of meals occurring.

(2) Effects of damage on amount and distribution of further grazing

(a) Amount eaten: The effect of damage on the amount eaten was not always clear-cut but was very distinct in Exp. (4) where much less feeding occurred on both classes of damaged twings than on the controls.

(b) Distribution: In the first 2 experiments the distribution of damage within leaves in terms of numbers of sites where feeding was initiated is very similar in all treatments. However, this conceals an important difference in the quality of these initiations; on artificially damaged treatments significantly more initiations are just small "nibbles" (% of the leaf area) than extended meals (5% of the leaf area). (χ^2=19.93, sig. at 0.1%). In the later experiments a greater number of initiations occur on the heavily damaged twigs but they are significantly smaller than those on the control twigs (χ^2=8.2, sig. at 1%).

No striking differences in foraging behaviour between twigs were noted in any of these experiments in terms of numbers of movements or numbers of meals eaten except that more movement occurred on the controls (cf. Edwards et al., 1988). However the date showed clear patterns of greater overall area eaten on the control twigs and also revealed that the caterpillars were biting the leaf at least as frequently on damaged leaves as control leaves but were often rejecting them after much less feeding. This suggests that there are real behavioural differences between caterpillars on different treatments but that the observations were not detailed enough to record them. Thus further experiments have been set up to record the foraging behaviour of larvae on damaged and undamaged leaves by video to allow detailed analysis.

REFERENCES

Edwards, P. J., S. D. Wratten and R. Gibberd (1988): The impact of inducible phytochemicals on food selection by insect herbivores and its consequences for the distribution of grazing damage. In: D. W. Tallamy and M J. Raupp. (eds), *Phytochemical Induction by Herbivores.* Wiley and Sons, New York.

Haukioja, E. and P. Niemelä (1976): Does birch defend itself actively against herbivores? *Rep. Kevo Subarct. Res. Sta. 13*, 44-47.

Schultz, J. C. (1983): Habitat selection and foraging tactics of caterpillars in heterogeneous trees. In: R. F. Denno and M. S. McClure (eds), *Variable Plants and Herbivores in Natural and Managed Systems.* Academic Press, New York. 61-90.

Silkstone, B. E. (1987): The consequences of leaf damage for subsequent insect grazing on birch (*Betula* spp.). A field experiment. *Oecologia 74*, 149-152.

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LEAF TRICHOMES AS INDUCIBLE DEFENSE OF THE GREY ALDER *ALNUS INCANA* (L.) AGAINST THE CHRYSOMELID BEETLE *AGELASTICA ALNI* L.

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Key words: induced defence, leaf surface, defoliation, herbivory, feeding preference, oviposition preference.

Induced resistance in the foliage of trees against herbivorous insects is often considered to be due to changes in concentrations of chemical plant compounds. Little is known about changes in physical properties of leaf surfaces, such as trichome density, in response to foliage damage by herbivores.

New leaves of the grey alder, *Alnus incana* (L.), emerge one after another from April to September during shoot elongation. Starting in May, old and damaged leaves fall off and therefore, a continuous leaf turnover results.

The alder beetle, *Agelastica alni* L., is oligophagous mainly on *Alnus* sp. Adults feed and oviposit (several batches) from late April to July. Larvae (3 instars) are the main cause of defoliation until pupation in August. Young adults feed for 2-4 weeks in early September before going into diapause.

Defense reaction of the tree. Different degrees of defoliation (0%-100% reduction of leaf area) were attained by a controlled release of alder beetles on randomly selected trees from a homogeneous population of three years old alders. The reduction in leaf area per tree and the density of leaf trichomes for a homogeneous age of leaves (7 days after unfolding) was assessed from June to September. Trichome density varied from hairless leaves with only sporadic hairs on veins to leaves with dense trichome layers on upper and lower surface (lower epidermis cells not visible). Highly significant positive correlations between trichome density and leaf area reduction, caused three weeks earlier, were obtained between July and September. Removal of the herbivores led to a decrease in trichome density of newly sprouting leaves.

Impact on the beetle. The influence of the trichome density on feeding and oviposition behaviour was analysed in dual-choice tests. Oviposition preference was tested with trichome-less vs. hairy leaves or two hairless leaves, respectively (control). To deposit their egg batches gravid females strongly preferred smooth leaves to hairy leaves, whereas no difference could be found between control leaves.

To exclude an influence of chemical plant compounds in feeding preference assays, parts of hairy leaves were tested against shaved parts of the same leaves. The pref-

erence for shaved parts was highly significant. No differences in the preference for shaved vs. unshaved parts of hairless control leaves could be found, proving that the shaving procedure itself did not change the attractivity of the leaves.

Foliage damage by *A. alni* in young grey alder trees enhances leaf trichome density of emerging leaves within three weeks of damage. Termination of herbivorous activity leads to a decrease in trichome density of newly sprouting leaves.

There is strong evidence for a negative influence of trichome density on feeding and oviposition behaviour of *A. alni*, forcing the mobile adults to migrate to undamaged trees and, concerning the less mobile larvae, reducing feeding efficiency and consequently increasing development time and mortality.

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EFFECT OF CARROT DEVELOPMENTAL STAGES ON OVIPOSITION BY *LISTRONOTUS OREGONENSIS* AND ITS EGG PARASITOID, *ANAPHES SORDIDATUS*.

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Key words: *Listronotus oregonensis*, *Anaphes sordidatus*, oviposition, egg parasitoid, carrot, searching efficiency, degree-days, sowing date.

In southwestern Quebec, the carrot weevil, *Listronotus oregonensis*, is univoltine and has an oviposition period ranging from 150 to 700 degree-days (base 7°C). Although carrot, which is its main cultivated host plant, is found at cotyledon stage as early as 150 degree-days when sown early, *L. oregonensis* will not oviposit until the carrot reaches the fourth true-leaf stage (Boivin, 1988). Therefore, late-sown carrots receive fewer eggs since they reach this stage toward the end of the oviposition period of *L. oregonensis*. Control of this pest should be synchronized with the development of the carrot on a field-per-field basis instead of a fixed number of degree-days.

Anaphes sordidatus, the only egg parasitoid of *L. oregonensis*, is present at the beginning of the oviposition period of *L. oregonensis* in carrot fields (Boivin, 1986). Modifications in the sowing date of the carrot to avoid most of the oviposition period of *L. oregonensis* could have an effect on the searching efficency of *A. sordidatus* and thus reduce the effectiveness of this control tactic.

Although *A. sordidatus* has been shown to have a positive functional response to the density of *L. oregonensis* eggs within a field during the season (Zhao, Boivin and Stewart; unpublished data), no relation was found between the date of sowing of carrots or the density of *L. oregonensis* eggs in a plot and the percentage of parasitism in that plot. This implies that carrot fields sown later to escape *L. oregonensis* oviposition period will have a lower egg density but not a lower parasitism rate. These results indicate that, within a field, *A. sordidatus* can respond to seasonal changes in egg density but that movement to other fields beyond its searching area is random and independent of egg density.

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REFERENCES

Boivin, G. (1986): *Anaphes sordidatus* (Girault) (Hymenoptera: Mymaridae), an egg parasitoid of the carrot weevil, *Listronotus oregonensis* (Le Conte). *Can. Entomol. 118*, 393-394.

Boivin, G. (1988): Effects of carrot developmental stages on feeding and oviposition of carrot weevil, *Listronotus oregonensis* (Le Conte) (Coleoptera: Curculionidae). *Environ. Entomol. 17*, 330-336.

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AIRBORNE INFORMATION TRANSFER BETWEEN COTTON PLANTS: CONSEQUENCES FOR PHYTOPHAGOUS MITES AND PREDATORY MITES

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Key words: *Tetranychus urticae*, *Phytoseiulus persimilis*, cotton, oviposition, airborne information, intrinsic defence, extrinsic defence, communication, contamination, attraction, olfaction.

Plant defence against herbivores may involve either an intrinsic action, such as the production of toxins, or an extrinsic action, such as attracting and/or arresting natural enemies of the herbivores (e.g. Price, 1986). There is evidence for such a mutualistic interaction between plants and predatory mites. Host plants release volatile chemicals in response to attack by phytophagous mites, such as spider mites, and predatory mites use these chemicals as cues in finding their prey (Dicke, 1988; Dicke and Sabelis, 1989).

In this study we investigated how unifested cotton plants (*Gossypium hirsutum* L., var. Acala SJ-2) respond to airborne chemical signals coming from nearby infested conspecifics.

Laboratory experiments were carried out, using a three-compartment windtunnel and a Y-tube olfactometer. The middle compartment of the windtunnel contained plants that were infested by spider mites. We measured the oviposition rate of female spider mites (*Tetranychus urticae* Koch) on uninfested plants that were exposed to the airborne signals (intrinsic defence). We also investigated the response of predatory mites (*Phytoseiulus persimilis* Athias-Henriot) towards odours coming from uninfested, but previously exposed cotton plants (extrinsic defence).

It was found that the oviposition rate of spider mites is lower on uninfested cotton plants that were exposed to airborne signals from infested plants, than on uninfested plants that were not exposed to such signals. Moreover, it was found that predatory mites are attracted olfactorily towards uninfested cotton plants that received airborne signals from spider mite infested plants.

From our experiments it can be concluded that uninfested, but exposed cotton plants induce a lower oviposition rate of the herbivore and elicit a response of the predator. These effects were caused by airborne **information transfer** from infested to uninfested plants. We argue that a distinction should be made between **communication** and **contamination**. Information transfer that induces an active response in the uninfested plants can be called communication, whereas contamination involves passive adsorption of volatile chemicals to the uninfested plants. Whether our find-

ings are due to an active response of the plant, triggered by airborne communication, or the consequence of passive contamination with airborne signals is a matter for future investigation.

REFERENCES

Dicke, M. (1988): *Infochemicals in tritrophic interactions: origin and function in a system consisting of predatory mites, phytophagous mites and their host plants*. Ph. D. Thesis, Agricultural University, Wageningen, The Netherlands, 235.

Dicke, M. and M. W. Sabelis (1989): How plants obtain predatory mites as bodyguards. *Neth. J. Zool.*, (in press).

Price, P. W. (1986): Ecological aspects of host plant resistance and biological control: interactions among three trophic levels. In: D. J. Boethel and R. D. Eikenbary (eds), *Interactions of Plant Resistance and Parasitoids and Predators of Insects*. Ellis Horwood Ltd., Chichester. 11-30.

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PROTEASE INHIBITORS AS RESISTANCE FACTORS AGAINST PASTURE PESTS

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Key words: *Costelytra zealandica*, *Wiseana* sp., *Teleogryllus commodus*, pest gut enzymes.

Pest insects. Major insect pests of pasture in New Zealand include grass grub beetle (*Costelytra zealandica*), porina caterpillar (*Wiseana* spp.) and black field cricket (*Teleogryllus commodus*). Grass grub larvae feed on white clover roots; crickets and porina feed on foliage of ryegrass and clover. Protease inhibitors are potential single gene resistance factors for pasture plants.

Procedure for identifying effective protease inhibitors. a) Pest gut enzymes were characterized by digestion of peptide substrate and by determination of pH optima, molecular weights and substrate response curves. b) Degree of enzyme binding by a range of inhibitors was determined in vitro. c) Protease inhibitors (PI) were incorporated into artificial diet at 0.1%, 0.33% and 1% by weight, and insect survival and growth rates were determined. Proteins with no inhibitory anti-enzyme activity were added to control diets at equivalent levels for comparison. d) Gut enzyme activity of test insects was measured at the end of the growth period.

Grass grub. Trypsin is the major digestive protease of *C. zealandica*. Wingbean, cowpea, potato-1 (POT-1), potato-2 and soybean (SBTI) trypsin inhibitors (TI) bound tightly to this enzyme in vitro, and reduced larval growth when incorporated into diet. Trypsin production was elevated in larvae feeding on SBTI. A combination of a carboxipeptidase (an exopeptidase) inhibitor from potato and SBTI (an endopeptidase inhibitor) reduced growth more than either of these alone.

Black field cricket. In *T. commodus*, the exopeptidases leucine amino peptidase and carboxypeptidases A and B showed high activity. Two endopeptidases, elastase and trypsin, were present in moderate and similar amounts. *In vitro* tests have identified several effective inhibitors including POT-1, alpha-1 antiproteinase, secretory leukocyte TI and pumpkin fruit PI against the elastase, and human inter-alpha TI, aprotinin (APRO) and wheatgerm TI-1 (WGI-1) against trypsin.

Porina caterpillar. Major gut enzymes in 2 species of *Wiseana* were trypsin and carboxipeptidase A. Effective trypsin inhibitors in vitro were arrowhead TI, WGI-1,

APRO, and to a lesser degree lima bean TI (LBTI) and SBTI. The proteases SBTI, LBTI, chicken and turkey eggwhite ovomucoids, the lectin concanavalin A and the non-inhibitory proteins casein, rubisco and bovine serum albumin were added to artificial diet. None of these significantly altered growth, survival, or enzyme activity.

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BRUCHUS-LATHYRUS RELATIONSHIPS: EFFECTS OF EXOGENEOUS HORMONES

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Key words: *Bruchus affinis*, *Lathyrus latifolius*, JH analogues, diapause, ecdysterone.

It is generally known that some crucial developmental events in the life cycle of *Bruchus affinis* appear to be synchronized and coordinated with growth, inflorescence and seed maturation in the host-plant, *Lathyrus latifolius* (for references see Labeyrie, 1990). The adults overwinter in a state of reproductive diapause which is terminated only when they feed on pollen of *L. latifolius* flowers next year. In this work, we have investigated some factors responsible for the adaptations of the insect to its host-plant. In analogy with other situations where developmental regulation is under neuroendocrine control, we have initiated these studies by analysis of the effects of exogenous juvenile hormone and ecdysterone. The diapausing adults of *B. affinis* were stored at +5°C in complete darkness. They were periodically removed in groups of 20 individuals and placed into plastic Petri dishes at 25°C, 16hr photoperiod, with drinking water and pollen (mixed pollen collected by honey bees). The selected analogues of JH (I = 11-chloro deritative of methyl farnesoate; II = phenoxycarb deritative W-328 from UOCHB; and III = tricyclic Sumitomo juvenoid S-71639) were applied to the substrate of the Petri dishes in ethanol; amounts in decimal scale from 0.01 to 100 $\mu g/cm^2$. The adults took up the tested compounds by contact with the treated substrate. Ecdysterone, 2 μg per insect, was injected into the abdomen in 10% ethanol. The effects were evaluated according to stimulation of ovarian growth, initiation of feeding, formation of excrements or by counting the deposited eggs.

We have found that:

1) Normal untreated females remain in diapause for more than 30 days at 25°C, 16hr photoperiod, without feeding;

2) Injections of ecdysterone have been also ineffective in breaking diapause under the above conditions;

3) Exposure to juvenoids terminated adult diapause, induced ovarian maturation and feeding;

4) Juvenoid compouds II and III stimulated ovarian growth in concentrations of 0.1 or 0.2 $\mu g/cm^2$, compound I was 50-100 times less effective;

5) Extensive experimental series with 1 $\mu g/cm^2$ of juvenoid III revealed stimulation of feeding on pollen from other plants than *L. latifolius* and deposition of eggs on substrates substantially different from the green pods of the host-plant;

6) The juvenoid–treated adults fequently showed mating activity, which was absent in diapausing specimens.

The above results show that we have really imitated all behavioural and developmental features associated with reproduction by exogenous application of JH analogues, in absence of the host-plant. As these features are always perfectly coordinted in nature by signals received from the plant, we conclude that the activities of *Bruchus affinis*, evoked by the presence of *Lathyrus latifolius,* can be viewed as an outcome of adaptations that are mediated by the neuroendocrine system.

REFERENCE

Labeyrie, V. (1990): Is it sufficient to consider plant-insect relationship to conclude about coevolution? In: J. Maynard-Smith and G. Vida (eds), *Organisational Constraints on the Dynamics of Evolution*. Manchester Univ. Press, Manchester. 373-383.

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FEEDING OF ACARID MITES ON DIFFERENT ORNAMENTAL PLANTS

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Key words: *Tyrophagus putrescentiae*, *Tyrophagus neiswanderi*, *Rhizoglyphus* sp., ornamental plants, narcissus, lily, hyacinth, tulip, freesia, crocus, hippeastrum, acarid mites.

Bulbs and corms of ornamental plants during storage for few months are very often infested by acarid mites. Since many years *Rhizoglyphus* species were known as common pests of corms of narcissus, lily, hyacinth, tulip and bulbs of freesia (Andison, 1951; Muller and Hollinger, 1980; Bielska, 1983). Although mites belonging to the genus *Tyrophagus* constitute a serious problem during storage of ornamental plant materials, only few data can be found in the literature on this problem (Wilkin et al., 1976; Czajkowska et al., 1988).

Degree of infestation and species composition as well as reproduction of *Tyrophagus putrescentiae* (Schrank) and *Tyrophagus neiswanderi* Johnston and Bruce on various species of ornamental plant materials were studied. Almost all samples (15 bulbs or corms in each sample) contained mites of the genera *Rhizoglyphus* and/or *Tyrophagus*. Thirty different varieties (12 tulip, 4 crocus, 4 hippeastrum, 3 freesia, 3 narcissus) were checked. The most numerous mites occurred on almost all varieties of tulip and freesia and on one crocus variety (Große Gelbe). On hippeastrum only singular colonies were found.

The reproduction rate of *T. putrescentiae* on 12 varieties of tulips was checked. The coefficient of population increase of *T. putrescentiae* was ranging from 2.9 (on Miss Triumph variety) to 999 (on Margared Herbst variety) during one month under storage conditions. The multiplication of *T. neiswanderi* was compared on 6 different host plants under laboratory conditions (temp. 25°C, rh 85%). 25 adult mites were put on one corm or bulb, all mobile stages were counted after 2 and 6 weeks. Number of mites increased distinctly after two weeks on tulip (to 140) and gladiolus (to 60). After six weeks further increase was recorded only on the same plant materials (300 on tulip and 120 on gladiolus). On the remaining 4 plants (hippeastrum, canna, narcissus, freesia) reproduction rate was very low both after 2 and 6 weeks.

REFERENCES

Andison, H. (1951): In: *Plant Protection in New Zealand*. Govt. Printer, Wellington. 446-447.

Bielska, I. (1983): Biologia i ekologia dwóch gatunków roztoczy z rodzaju *Rhizoglyphus – R. echinopus* (F. i R.) i *R. robini* Clap. *Zeszyty Problemowe Nauk Rolniczych*, 95-123. (In Polish.)

Czajkowska, B., M. van de Vrie and D. Kropczynska (1988): Mites of the genus *Tyrophagus* as pests of ornamentals in greenhouses. *Med. Fac. Landbouww. Rijksuniv. Gent*, 53/2b.

Muller, P. J. and T. C. Hollinger (1980): Damage by *Rhyzoglyphus* mites in some ornamental bulbous crops. *Acta Horticulturae, 109*, 449-456.

Wilkin, D., G. Murdoch and H. C. Woodville (1976): The chemical control of mites infesting freesia corms and narcissus bulbs. *Ann. Appl. Biol. 82*, 186-189.

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HOST PLANT OVIPOSITION PREFERENCE OF THE EUROPEAN CORN BORER (*OSTRINIA NUBILALIS* HBN.). A BIOCHEMICAL EXPLANATION

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Key words: oviposition preference, host-plant, maize, sunflower, phylloplane, leaf surface, carbohydrates, amino acids, *Ostrinia nubilalis*, Lepidoptera.

We proposed some biochemical explanation for the contact stimuli effective after postalighting on the leaves when European corn borer (ECB) (*Ostrinia nubilalis* Hbn.) females had the choice between several corn hybrids or between differently modified plants of one corn hybrid (Derridj et al., 1986).

A high correlation between low molecular carbohydrate levels inside the leaves and the insect preference was observed (Fiala et al., 1985). The study was carried out 1) to determine if these substances may be present on the leaf surface and consequently act directly on insect behaviour, and 2) to know if the phenomenon observed on the corn could be extended to the insect's interspecific plant preference and to other substances like free amino acids.

All experiments were pursued on whole corn and sunflower plants, cultivated hydroponically on vermiculite. The phenology of experimental plants varied from young stages to flowering. The bioassays with insects were carried out in green house compartments and plants were given in binary choices. The oviposition preference was estimated by the number of egg masses laid.

Leaf surface substances for analysis were collected by spraying the leaves for 30 sec with ultrapure water.

The results with corn and sunflower leaves showed that low amounts (10^{-5} to 10^{-6} moles.m^{-2} of leaf surface) of *glucose, fructose, sucrose* and of free *amino acids* were collected by this technique. Their quantities varied with the plant species, varieties, their phenological stage and with leaf position. The relative amount of an individual substance in each sample was rather stable for the carbohydrates, while it was very specific for the amino acids. Thus, nutrients are present on the plant surface and can be contacted by the insect.

The intraspecific and the interspecific oviposition preference between corn and sunflower were highly correlated with the relative differences in quantities of fructose and in other sugars collected on the two plant components of the choices given to ECB. Proline accentuated these effects. The preference was proportional to the differences in fructose level (Derridj et al., 1989).

Statistical analyses were carried out exclusively on the substances (fructose, glucose, sucrose, proline) by the PRINCOMP (principal component analysis). The plants, according to their biochemical composition, were grouped.

No differences in quantities of substances on the leaf surface correlated with non preference of the insect. However, if similar data were used only for those plants for which marked preference was shown, quantitative traits correlated suitably with preference. These results were partly corroborated by those obtained in experiments with filter paper strips in which these substances were incorporated.

Other methods of detection and localization of the organic molecules on the plant surface and experiments on artificial supports will be used to improve our knowledge of insect behaviour.

REFERENCES

Derridj, S., V. Fiala and E. Jolivet (1986): Increase of European corn borer (*Ostrinia nubilalis*) oviposition induced by a treatment of maize plants with maleic hydrazide. Role of leaf carbohydrate content. *Entomol. exp. appl. 41*, 305-310.

Derridj, S., V. Grégoire, J. P. Boutin and V. Fiala (1989): Plant growth stages in the interspecific oviposition preference of the European corn borer and relation with chemicals present on the leaf surface. *Entomol. exp. appl. 53*, 267-276.

Fiala, V., S. Derridj and E. Jolivet (1985): Influence de la teneur en glucides solubles des feuilles de *Zea mays* L. sur le choix du site de ponte de la pyrale *Ostrinia nubilalis* Hbn. (Lepid., Pyralidae). *Agronomie 5*, 927-933.

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THE JOINT EFFECT OF AN ANTIFEEDANT AND STRESSED HOST-PLANTS ON THE DEVELOPMENT AND BEHAVIOUR OF THE COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA*

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Key words: water-stress, insect damage, copper-sulphate, potato, larval, prepupal and adult weights, young and old foliage.

Different kinds of stresses may influence the susceptibility of plants to insect attack (Bernays and Lewis, 1986). Naturally occurring water or nutrient stress, disease and previous damage, etc. on the host-plant have all been reported to increase or decrease feeding or development of different insect species (White, 1976; Crawley, 1983). Any additional treatment (such as applications of antifeedants) on stressed plants may facilitate the behavioural or physiological effects on the consumers.

To my knowledge, for the first time, the present work tries to report on the joint effects of variously stressed plants and an antifeedant on the behaviour and development of the Colorado potato beetle.

Plants were stressed by watering (under- and over-watering), by insect damage and by a combination of the two. Green-house experiments were carried out using potted green-house-grown potato. Eight replicates were used for each treatment (stressed plants without and with 0.05 M copper sulphate), and control, resp. Equal numbers of newly emerged, unfed, 2nd instar, laboratory-reared Colorado potato beetle larvae were introduced into light nylon sleeve-cages placed over the plants. In addition acceptability of young and old foliages by adults and larvae was also examined in preference tests. Evaluations were done on the bases of larval, prepupal and adult weights, length of development, number of eggs laid on control plants even after exposure of adults to stressed plants. For the preference tests (choice-situation with alternately arranged disks) statistical analysis was done by using the discrimintion quotient (DQ) (David and van Herrewege, 1970).

In general, feeding on stressed plants decreased 4th larval instar-, prepupal- and adult weights. The reduction was greater with instars reared on stressed+antifeedant-treated plants than on stressed ones alone. A drastic effect on larval feeding and on the palatability of the plant was induced with insect damage stress. Significant interactions were shown between stress and antifeedant applications on the developmental period. It was also found that the production of eggs/plant is influenced by both water stress (under- and over-watering) and insect damage, other stresses showing nonsignificant differences from control. Survival of neonate larvae was

significantly (DNMR-test, P = 1%) affected only by feeding on foliage having exposed to insect consumption previously. The preference tests showed that young leaves were preferred to old ones both on control and treated plants (both by larvae and adults).

The results obtained here, i.e. the retarded growth of larvae may be the result of active defence reactions from the plant side, for instance, the result of chemical changes activated by defoliation, a process similar to the one found by Haukioja and Niemelä (1977). Bernays and Lewis (1986) also hypothesized that changes occurring during wilting, such as increases in the concentrations of amino acids, may be involved in the palatability change.

REFERENCES

Bernays, E. A. and A. C. Lewis (1986): The effect of wilting on palatability to *Schistocerca gregaria*, the desert locust. *Oecologia 70,* 132-135.

Crawley, M. J. (1983): *Herbivory. The Dynamics of Animal-Plant Interactions*. University of California Press, Berkeley.

David, J. and J. van Herrewage (1970): Choix d'un site de ponte chez *Drosophila melanogaster*: technique d'étude et variabilité. *Rev. Comp. Anim. 4,* 82-84.

Haukioja, E. and P. Niemelä (1977): Retarded growth of a geometrid larva after mechanical damage to leaves of its host tree. *Ann. Zool. Fennici 14,* 48-52.

White, T. C. R. (1976): Weather, food and plagues of locusts. *Oecologia 22,* 119-134.

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THE ROLE OF SECONDARY PLANT METABOLITES IN HOST-LOCATION BY THE CABBAGE SEED WEEVIL, *CEUTHORHYNCUS ASSIMILIS* PAYK

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Key words: *Brassica napus*, olfactometer, electroantennogram, sabinene.

The response of cabbage seed weevils (*Ceuthorhyncus assimilis* Payk.) to the odour of oilseed rape (*Brassica napus* L.) was studied using an olfactometer and by electroantennograms (EAG's).

The olfactometer (based on a design by Vet et al., 1983) presents the weevils with four sources of odour. In this study, the odour to be tested is presented from one source only; the three others being filtered air.

The responses are classed into "definite choices" if the insect moves to the source of the odour, and "provisional choices" if the insect remains in the odour plume but does not move to the odour source.

Male seed weevils are significantly attracted to the odour of rape flowers (definite choice, $p<0.001$) and female weevils exhibit a significant provisional choice for rape flower odour ($p<0.001$). Chemical analysis of rape flower odour uncovered several compounds including a monoterpene; sabinene (Evans unpublished; Tollsten and Bergström, 1988), and this compound was used in further olfactometer studies (Table 1). Female weevils are significantly attracted to sabinene down to a concentration fo 1% whereas males are only attracted to 5% sabinene (Table 1).

Table 1 % Response of male and female cabbage seed weevils to sabinene (a compound present in rape flower odour) in an olfactometer.

Concentration of sabinene	Sex of weevil	Odour stimulus				
		Blank	Blank	Blank	Sabinene	p*
5%	M	17.67	21.43	24.06	36.84	<0.001
	F	20.53	22.63	22.63	34.21	<0.001
2.5%	M	23.27	24.53	23.90	28.30	n.s.
	F	22.85	22.52	21.85	32.78	<0.001
1%	M	21.52	20.86	26.82	30.79	n.s.
	F	19.03	26.01	21.45	33.51	<0.001

* Significance level of χ^2 analysis of definite choices by seed weevils carried out on raw data not % response.

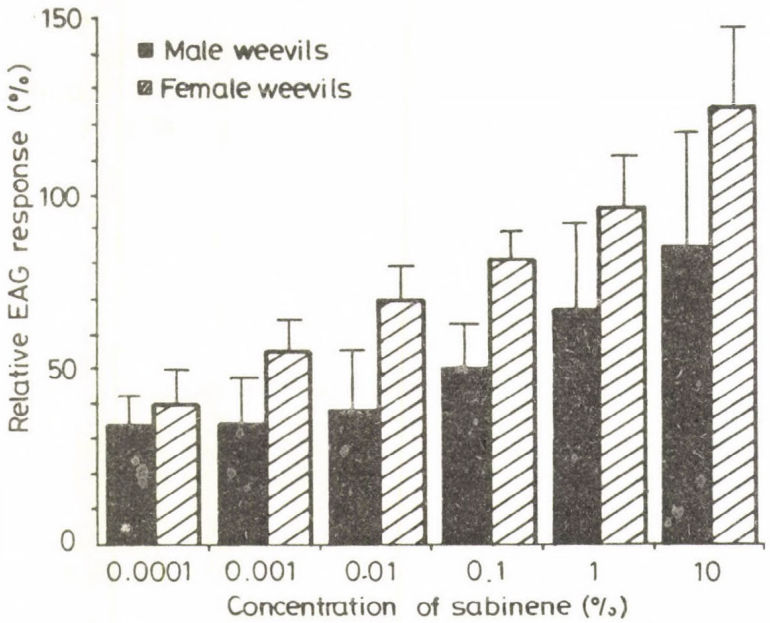

Fig. 1 EAG responses of male and female *Ceuthorhyncus assimilis* to sabinene; a compound present in rape flower odour. Mean EAG responses as a percentage of the response to 1% cis-3-hexen-1-ol. Sabinene and cis-3-hexen-1-ol were diluted in hexane. Vertical lines indicate standard error of the means (n = 6). Significant difference between male and female weevils in their response to sabinene ($p<0.001$, Analysis of variance).

EAG studies (see Visser, 1979 for methodology) indicates a stronger dose-response to sabinene by female seed weevils (Fig. 1). The behavioural responses of seed weevils to sabinene in the olfactometer are supported by the response to sabinene at the sensory level.

This approach is being applied to other compounds present in rape flower and leaf odour.

REFERENCES

Tollsten, L. and G. Bergström (1988): Headspace volatiles of whole plants and macerated plant parts of *Brassica* and *Sinapis*. *Phytochemistry 27,* 4013-4018.

Vet, L. E. M., J. C. van Lenteren, M. Heymans and E. Meelis (1983): An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. *Physiol. Entomol. 8,* 97-106.

Visser, J. H. (1979): Electroantennogram responses of the Colorado beetle *Leptinotarsa decemlineata* to plant volatiles. *Entomol. exp. appl. 25,* 86-97.

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OVIPOSITION PREFERENCE AND LARVAL FOOD QUALITY IN *HELIOTHIS ARMIGERA*

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Key words: biological fitness index, tomato, lucerne, aster, sweetcorn.

Heliothis armigera (Hübner) is an occasional pest in New Zealand of a range of cultivated plants including tomato (*Lycopersicon esculentum*), sweetcorn (*Zea mays*), lucerne (*Medicago sativa*) and flower crops such as aster (*Callistephus chinensis*). Larvae feed mostly on the reproductive parts of plants (flowers, fruits) but foliage injury also occurs especially on lucerne, which unless grown for seed is not managed to permit flowering.

The nutritional value of the above named plants to *Heliothis armigera* was evaluated by feeding plant material *ad libitum* to groups of laboratory reared larvae from egg hatch through to adult emergence and recording the following parameters: duration of larval period, percentage pupation, duration of pupal period, weight of pupae, percentage emergence to adults, fecundity of adult females, and percentage egg hatch. For each plant species, with the exception of lucerne, separate groups of larvae were fed foliage and reproductive parts (flowers, fruit, seed head (corn cob) as appropriate). A kidney bean based artificial diet was included as a basis of comparison.

From the above parameters a biological fitness index was derived for each diet using the following formula:

$$\text{biological fitness index} = \frac{lx\ ex\ hx}{1000\ Tl\ Tp}$$

where lx = % pupation, ex = fecundity, hx = % egg hatch, Tl = larval duration
Tp = pupal duration

Index values relative to artificial diet = 1.0, and ranking of the diets are set out in Table 1.

Oviposition preference tests were conduced by allowing free flying adult moths to select oviposition sites on whole potted plants within flight cages. Two series of tests were conducted. In one series only a single plant species (mature flowering plants) was offered at a time (no-choice tests) so that selection was of part of the plant on which eggs were laid. In the second series the four species of plants were

Table 1 Biological fitness index values of different foods for *Helitohis armigera*, relative to artificial diet=1.00

food	biological fitness index	rank
artificial diet	1.00	2
lucerne leaves	0.27	5
tomato leaves	0.06	7
tomato fruits	1.14	1
aster leaves	0.24	6
aster flowers	0.74	4
sweetcorn leaves	0.06	8
sweetcorn cobs	0.91	3

Table 2 Oviposition preference by *Helitohis armigera* for plant parts in non-choice tests with single plant species

Plant part	Percentage of eggs laid			
	Lucerne	Tomato	Sweetcorn	Aster
Leaves	82.0	75.9	70.4	54.0
Stems	4.8	2.4	2.6	29.5
Flowers and flower buds (includes tassels, i.e. male flowers of sweetcorn)	13.2	16.7	10.4	16.5
Fruits (includes cobs of sweetcorn)	-	5.0	16.6	-

All values in each column are significantly different from each other (p=0.05)

offered simultaneously (choice tests). Separate tests were conducted with immature (non-flowering) and mature (in flower and fruits forming) plants. The results of the single species (no-choice) tests are shown in Table 2.

Choice tests between species gave the following order of preference: for immature plants: - lucerne > tomato = aster >sweetcorn, for mature plants: aster > sweetcorn > tomato > lucerne.

Species preference was altered markedly when mature flowering plants were offered compared to immature non-flowering plants.

The results indicate that within plant species there is a strong preference for oviposition on foliage compared to other plant parts despite the fact that dietary evaluation showed foliage to be much less nutritious for all plant species than reproductive parts. Similarly, oviposition preference between species was not consistent with nutritional value for larvae either for immature or mature plants. The question must therefore be raised whether there is some valid ecological explanation for these apparent anomalies [see Thompson (1988) for relevant discussion] or whether the results are a consequence of artificial conditions of experimentation. Field based studies should help to elucidate this.

REFERENCE

Thompson, J. N. (1988): Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomol. exp. appl. 47,* 3-14.

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DIFFERENTIAL ANTENNAL SENSITIVITY TO HOST ODOR COMPOUNDS IN *RHAGOLETIS* SIBLING SPECIES (DIPT.: TEPHRITIDAE)

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Key words: antennal sensitivity differences, host odor compounds, fruit extracts, *Rhagoletis pomonella, Rhagoletis mendax*, sibling species, host finding, electroantennogram, gas chromatogram, splitter.

Host odor is among the primary cues in host finding and acceptance in *Rhagoletis* species (Prokopy et al., 1973). Electroantennogram (EAG) responses to esters known to contribute to the odor of apples have been shown to correspond to behavioral host finding responses in *R. pomonella* (Fein et al., 1982). This indicates that host odor perception through the antenna is a primary cue in host finding in this species. We recently established differences between the closely related sibling species *R. pomonella* and *R. mendax* in EAG response to odors of their respective host fruits, apple and blueberry (Frey, J.E. and G.L. Bush, manuscript). These data indicate that *R. mendax* is relatively more sensitive to the odor of blueberries, its original host, than *R. pomonella* whose main hosts are hawthorn and apple. To study the underlying sensitivity differences we analyzed the sensitivity spectrum of both species to apple and blueberry odors using a gas chromatograph with an output split between a flame ionization detector and an antennal preparation as electroantennogram detector.

We used mature females of both species collected as pupae from their respective host plants (apples from Hart, Michigan; blueberries from Sawyer, Michigan). Headspace odours of blueberries and apples were prepared as pentane washes of whole fruits. For the analysis of interspecific differences, we used the average rank of the nine EAG responses with the largest average amplitude for both species in response to each host fruit extract.

Mean rank difference between the species is 73.5 for apple and 162.0 for blueberry fruit extract indicating that interspecific peripheral sensitivity differences are significant for both host fruit extracts but stronger for blueberry ($F=3.235$, $p<0.001$) than for apple ($F=3.173$, $p=0.001$) extract.

These data indicate that interspecific differences in antennal sensitivity between *R. pomonella* and *R. mendax* are at least partly due to differences in response to a number of single, mainly low molecular weight odour compounds of host fruit extracts. Identification of some of these odour compounds is under way and bioassays

will reveal the behavioural significance of the peripheral sensitivity differences between *R. pomonella* and *R. mendax*.

REFERENCES

Fein, B. L., W. H. Reissig and W. L. Roelofs (1982): Identification of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella*. *J. Chem. Ecol. 8*, 1473-1487.

Frey, J. E. and G. L. Bush: *Rhagoletis Sibling Species and Host Races (Diptera: Tephritidae) Differ in Host Odor Recognition.* Manuscript.

Prokopy, R. J., V. Moerike and G. L. Bush (1973): Attraction of apple maggot flies to odor of apples. *Environ. Entomol. 2*, 743-749.

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ULTRASONIC ACOUSTIC EMISSIONS FROM DROUGHT-STRESSED TREES: IMPLICATIONS FOR BARK- AND WOOD-FEEDING INSECTS

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Key words: ultrasound, acoustic emissions, drought stress, xylem, *Ips*, bark beetles, Coleoptera, Scolytidae, pine, *Pinus*.

Bark beetles (Scolytidae) prefer to attack drought-stressed trees, but just how they recognize them is not completely understood (Mattson and Haack, 1987). Visual and olfactory cues are undoubtedly important. Ultrasonic acoustic emissions (AEs) from stressed trees may also be involved. AEs were first reported in 1983 and result from the energy released when individual water columns break within the tree's conducting xylem (Tyree and Sperry, 1989).

Objectives were to characterize the AEs from drought-stressed white pine trees and then determine if *Ips* bark beetles prefer to colonize trees or branches that are pulsed with ultrasound that simulates the drought condition.

Water is withheld from potted pines to induce drought stress. AEs are monitored between 40-1000 kHz during an extended period of drought by attaching sensitive transducers to the trunks. The signals are amplified and analyzed to determine signal rate, duration, strength, and frequency. Early results appear in Haack et al. (1988).

The second phase of the study is currently under way and involves pulsing potted trees or branch material with ultrasound at settings that simulate various degrees of drought stress. Bark beetles are placed in a test chamber and allowed to choose between two trees or two branch samples, similar except that one is being pulsed with ultrasound while the other serves as a "silent" control. Colonization behavior is recorded after 24 hours.

Drought-stressed pines produced abundant AEs after 2-3 weeks without water, while well-watered control trees were mostly silent. Initially, AE rate increased with increasing degree of water stress, peaking at about 2-3 AEs per second. Others have obtained similar results (Tyree and Sperry, 1989).

In an earlier study (Haack et al., unpublished data), where male *Ips* were given a choice between AE-producing branches (cut and allowed to dry) and "silent" branches (cut but end-dipped in vax to slow drying and thus AE production), 36 males tunneled into the pulsed branches while only 7 entered the "silent" branches. Such results suggest that AEs may play some role in host acceptance.

Results of the ultrasonic pulsing experiments are not yet available. Some technical difficulties still exist in the design of the bioassay chamber and in generating an AE signal that is broadly similar throughout the portion of trunk or branch that is exposed to the beetles.

REFERENCES

Haack, R. A., R. W. Blank, F. T. Fink and W. J. Mattson (1988): Ultrasonic acoustical emissions from sapwood of eastern white pine, northern red oak, red maple, and paper birch: Implications for bark- and wood-feeding insects. *Florida Entomol. 71,* 427-440.

Mattson, W. J. and R. A. Haack (1987): The role of drought in outbreaks of plant-eating insects. *Bioscience 37,* 110-118.

Tyree, M. T. and J. S. Sperry (1989): Vulnerability of xylem to cavitation and embolism. *Annu. Rev. Plant Physiol. 40,* 19-38.

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THE IMPACT OF SHORT- AND LONG-TERM INDUCIBLE DEFENCES OF MOUNTAIN BIRCH ON ITS LEAF-CHEWING SAWFLY SPECIES

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Key words: induced defence, *Betula pubescens* ssp. *tortuosa*, *Epirrita autumnata*, sawflies, relative growth rate, competition.

Insect herbivores are thought not to compete for food resources (Lawton and Strong, 1981). However, inducible defence reactions in host plants, either rapidly inducible short-term (RIR) or delayed long-term inducible reactions (DIR) (Haukioja et al., 1988), may interfere with herbivore species interactions (Neuvonen et al., 1988). I studied the seasonal occurrence and persistence of induced defence reactions in mountain birch, *Betula pubescens* ssp. *tortuosa* (Ledeb.) Nyman, by testing their impact on the growth of eleven leaf-chewing sawfly species at the Kevo Subarctic Research Institute in Finnish Lapland.

I had three treatments, each consisting of ten birch trees: 1) 50 % of the leaf biomass was torn early in the previous season and autumnal moths, *Epirrita autumnata* (Bkh.) (Lep.: Geometridae), were feeding early in the current season (= DIR + RIR),
2) autumnal moths were feeding in the current season (= RIR), and
3) trees without any damage (= Control). The leaf quality of the experimental trees was assessed by measuring the relative growth rates of larvae of different sawfly species over 24 h.

Leaf damage simulating the feeding of early season insect hebivores on mountain birch affected the growth of the tested sawfly species. In general, early and mid-season species were more strongly affected by induced reactions than late-season species. It is supposed that seasonal deterioration of leaf quality either masks the effects of induced defences or late-season species are better adapted to low-quality food. The growth of the larvae of mid-season sawfly species was affected by both short- and long-term induced reactions. This result indicates that early season species may escape short-term induced reactions of mountain birch in current year but may not avoid long-term effects. Some species showed variation in their responses to induced defences between years. This may be due to yearly differences in induced reactions as well as due to species-specific responses.

Induced defence reactions may have an important role in competitive interactions between herbivore species, but the impact of these defence reactions should be studied with long-term experiments.

REFERENCES

Lawton, J. H. and D. R. Strong (1981): Community patterns and competition in folivorous insects. *Am. Nat. 118,* 317-338.

Neuvonen, S., S. Hanhimäki, J. Suomela and E. Haukioja (1988): Early season damage to birch foliage affects the performance of a late season herbivore. *J. appl. entomol. 105,* 182-189.

Haukioja, E., S. Neuvonen, S. Hanhimäki and P. Niemelä (1988): The autumnal moth in Fennoscandia. In: A. A. Berryman (ed), *Dynamics of Forest Insect Populations: Patterns, Causes, and Management Strategies*. Plenum Press, New York. 166-178.

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ACTION OF SOME DERIVATIVES OF PHYTOECDYSONES ON INSECTS

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Key words: Phytoecdysones, ecdysterone, 5β- hydroxy-ecdysterone, apolar ecdysteroid derivatives, fatty acid esters, *Sarcophaga* test, ecdysone activity unit (SU), topical effect, *Sarcophaga bullata, Galleria mellonella*

Ecdysteroid compounds with hormonal activity in insects occur in a large number of taxonomically unrelated plants. There exist long lasting disputes concerning the ecological significance of these phytoecdysones in insect-plant interactions. It has been noticed that plants contain sometimes larger concentrations of ecdysteroids, compared with their dietary concentrations effective in the oral assays. However, several ecdysteroid conjugates have been found in plants, whose biological activity in insects remains still unknown. In the excrements of insects there are apolar metabolites represented by various fatty acid esters of ecdysteroids (Connat and Diehl, 1986). In plants such apolar ecdysteroid conjugates have not been detected so far. However, this might be eventually due to their unknown biological activity in previous screening assays. In order to know whether they are active, we have prepared several fatty acid esters of 20-hydroxyecdysone (1) and 5β, 20-dihydroxyecdysone (2) and tested their hormonal activity. Palmitic and oleic anhydrides were used for preparation of 2-palmitoyl and 2-oleoyl monoesters (3-6). For preparation of di- and triesters (7-10) stearoyl chloride was used. Our attention has been extended also to some glycosidic conjugates, which are known to occur in plants. Silenoside A (11) from *Silene brachuica* (provided us by Saatov et al., 1981) was applied.

The compounds 1-11 were tested in the standard injection assay using ligated larval abdomens of *Sarcophaga bullata* and *Galleria mellonella*. In addition, the compounds were subjected to extensive topical assays, by applying on the body surface of ligatured larvae of *Dermestes vulpinus, Manduca sexta, S. bullata* and *G. mellonella*, on last instar larvae of *Pyrrhocoris apterus* and on pupae of *Tenebrio molitor*.

The results obtained in the standard injection assay on *Sarcophaga* are summarized in Table 1. They show that the 2-palmitoyl-(3,4) and 2-oleoyl-(5,6) monoesters have been as active as the parent ecdysteroides 1 and 2.

Similar, though yet incomplete results have been obtained in the injection assays on ligated larvae of *Galleria*. On the other hand, the ecdysterone distearoyl esters 7-9 and 2,3,22-tristearoyl ester 10 as well as the 22-O-α-D-galactoside 11 were biologically inactive. In topical assays all compounds 1-11 were inactive in all species

Table 1 The ecdysone activity units in standard *Sarcophaga* test (SU), i.e. amounts in μg, effecting 50% of stimulations at the formation of puparium

compound	1	2	3	4	5	6	7	8	9	10	11
SU [μg]	0.25	0.7	0.25	0.25	0.4	5	i	i	i	i	i

Note: i=inactive up to 20μg of injected compound

and stages tested. This shows that if the plants contained similar inactive derivatives, as shown in Table 1, they could indeed escape the attention in earlier screenings.

REFERENCES

Connat, J. L. and P. A. Diehl (1986): Probable occurrence of ecdysteroid fatty acid esters in different classes of arthropods. *Insect Biochem. 16,* 91-97.

Saatov, Z., M. B. Gorovitz, N. D. Abdullayev, B. Z. Usmanov and N. K. Abubakirov (1981): Phytoecdysteroids from the plants of genus *Silene*. III. Silenosid A — a new glycosidic ecdysteroid from *Silene brachuica*. *Khimia Prirod. Soedin.* 738-744. (In Russ.)

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RESISTANCE OF LETTUCE TO THE APHID *NASONOVIA RIBISNIGRI.* Are Electrical Penetration Graphs (EPGs) helpful to find the origin of resistance?

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Key words: electrical penetration graph (EPG), aphids, *Nasonovia ribisnigri*, lettuce, resistance.

EPG recording of aphid penetration activities shows many different wave patterns some of which have been correlated with the position of the stylets in the plant (Tjallingii, 1985, 1989). So far little is known about the activities of the aphid during penetration, especially before the phloem is reached (mainly pattern C). It is still unknown whether and (if so) where the aphids ingest small volumes of plant material before the phloem is reached. On artificial diet a small intake of radioactive diet during ABC pattern was shown by Tjallingii (1978). During pattern ABC many intracellular punctures do take place which are recorded as potential drops (pd) (Tjallingii, 1985).

EPG recordings were performed as described by Tjallingii (1985) with a DC amplifier. Alate females of *Nasonovia ribisnigri* were used for EPG recording. After the gold wire was attached to the aphid it was placed on a Taiwan plant for at least 3 hours. Then it was transferred to the susceptible or resistant lettuce were the EPG was recorded during 3 hours.

On lettuce the frequency and summed durations of the EPG patterns differ between resistant and susceptible genotypes (Fig. 1). During a 3-hour recording the number of penetrations is higher on the resistant cultivar, the penetrations are shorter and less aphids reach the phloem than on the susceptible cultivar. From access to the plant (start of the EPG recording) it takes more time for aphids to reach a sieve element on the resistant line. Yet, seen from the start of the "succesful" penetration the aphid is able to reach the sieve element on the resistant line as fast as on the susceptible genotype.

Nasonovia ribisnigri is able to discriminate between resistant and susceptible lettuce genotypes from clues perceived *before* a sieve element is reached. These differences are probably not caused by mechanical barriers since the sieve elements can be reached equally fast on both lines. Experiments should focus on the correlation between EPG patterns, the exact position of the stylets during the EPG and the possible intake of small volumes of sap which can be tasted by the pharyngeal chemoreceptors during the ABC pattern. Which cells are punctured by the aphid,

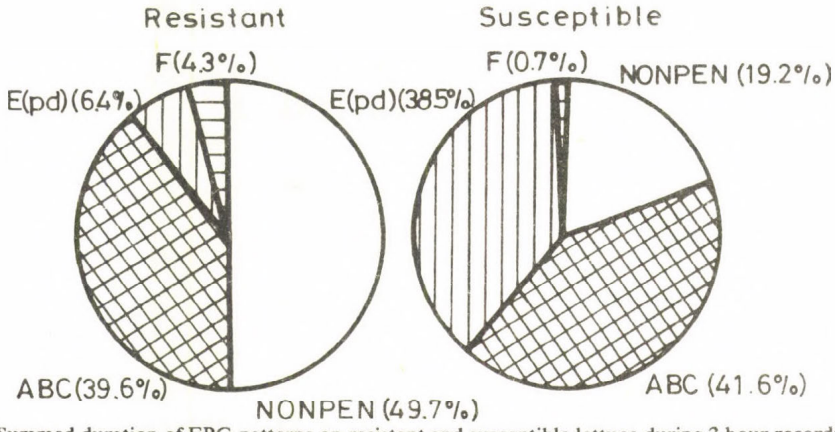

Fig. 1 Summed duration of EPG patterns on resistant and susceptible lettuce during 3 hour recordings. Patterns: ABC=A, B and C together; E (pd)=E2; F1=F; NONPEN= non penetration time.

does it ingest food from these cells? Simultaneous recordings of EPGs and myograms of the food-pump muscles and/or radio-isotope experiments will be tried to get more information. When a chemical substance correlated with resistance can be found, the position of this substance in the plant can provide new clues on aphid behaviour. Other causes of resistance (plant volatiles or non chemical stimuli) still can not be excluded.

REFERENCES

Tjallingii, W. F. (1978): Electronic recording of penetration behaviour by aphids. *Entomol. exp. appl. 24*, 721-730 .

Tjallingii, W. F. (1985): *Stylet penetration activities by aphids.* Thesis Agricultural University Wageningen, The Netherlands.

Tjallingii, W. F. (1989): Continuous recording of stylet penetration activities by aphids. *XVIII International Congress of Entomology. Proceedings Symposium "Mechanisms of Aphid-Plant Genotype Interactions".* (in press)

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FINE STRUCTURE OF APHID STYLETS IN PLANT TISSUE

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Key words: electrical penetration graph, *Aphis fabae*, serial sectioning, stylet pathway, transmission electron microscopy.

Aphid-plant interactions at cellular and subcellular levels need the resolution of Transmission Electron Microscopy (TEM). Earlier TEM studies, of mainly transverse sections of inserted stylets (Kimmins, 1986; Kimmins and Tjallingii, 1985), have already indicated aspects of the process of stylet penetration in plants that were not apparent from light microscopic research (Pollard, 1973). Thus it was demonstrated that stylets can pass through cells while remaining outside the plasmalemma, i.e. intramurally (Spiller et al., 1985).

When the stylets of a feeding aphid are amputated by microcautery the tips of the stylets can be located subsequently inside the plasmalemma of a sieve tube by TEM. The sieve tube cell apparently remains alive during sap ingestion by the aphid, as is demonstrated by the continuous trans-membrane potential that can be recorded in Electrical Penetration Graphs (EPG, Tjallingii, 1988). These EPGs also show many short intracellular punctures during pathway activities en route from epidermis to sieve elements.

In the study reported here, EPG recording and stylet amputation was combined with **serial** sectioning for TEM. Sectioning longitudinal to the direction of the stylets and transverse to the leaf vein enabled a reconstruction of a complete stylet pathway in relation to each vein element.

Apterous virginoparous adults of *Aphis fabae* were reared on *Vicia faba* in the greenhouse. An EPG was recorded until a sustained wave-form pattern E2 (formerly labelled as E(pd)) had indicated passive ingestion from a sieve element (Kimmins and Tjallingii, 1985) and the leaf tissue with the inserted stylets was then processed for serial TEM. Five sections of about 80 nm were mounted on each grid, and about 150 grids were needed to include the complete stylet pathway.

The EPG of an early penetration by *Aphis faba*, previous to the one which was amputated and sectioned, shows alternating periods of pathway activity (pattern C) including numerous short punctures (pd), and periods of sieve element puncturing (pattern E1 and E2), or of xylem ingestion (G). The sectioned penetration ended with sustained pattern E2, indicating that at least one sieve tube was punctured.

Table 1 Numbers of cells in and outside the vascular bundle which are involved in stylet penetration.

cell type	in vascular bundle					outside bundle		total & outside
	SE	CC	XE	VP	Total	MC	EC	
number of cells	12	10	13	40	75			
cells touched	11	6	3	21	41	22	2	65
cells with break in wall	11	4	3	17	35	19	2	56
break/touched cell	2.1	4	3	2.5		0.7	1	
cells damaged	2	2			4		1	5

TEM micrographs of the ultrathin sections could be combined to give a two dimensional projection of the complete stylet pathway including one track containing the stylets and a number of empty side branches which had been followed earlier in the same penetration. The composite overview reflects a 'vertical' distance of about 100 grids, i.e. 500 x .08 = 40 μ, from the epidermal insertion point to the xylem puncture. The stylets have 'touched' a large number of cells in different tissues (i.e. at least the sheath has made contact with the cell walls). These contacts left clear traces of breaks through some cell walls, but only a few cells had been damaged (i.e. become necrotic) - see summary in Table 1. Only one sieve element in the whole vein was not touched and punctured. Also a large proportion of the companion and vascular parenchyma cells has been touched and shows breaks. We often found more than one break in a cell wall (breaks/touched cell, Table 1). This number seems higher in companion cells than in sieve elements. Only a part of the breaks could be detected however, some were hidden behind grid bars, they cannot always be well recognized when transversely sectioned, and micrographs have been made of only a part of the available sections. No particular relation was found between branch endings and cell punctures. Correlating EPGs with TEM needs more research on much shorter, less complicated penetrations.

REFERENCES

Kimmins, F. M. (1986): Ultrastructure of the stylet pathway of *Brevicoryne brassicae* in host plant tissue, *Brassica oleracea*. *Entomol. exp. appl. 41*, 283-290.

Kimmins, F. M. and W. F. Tjallingii (1985): Ultrastructure of sieve element penetration by aphid stylets during electrical recording. *Entomol. exp. appl. 39*, 135-143.

Pollard, D. G. (1973): Plant penetration by feeding aphids (Hemiptera, Aphidoidea): A review. *Bull. Entomol. Res. 62*, 631-714.

Spiller, N. J., F. M. Kimmins and M. Llewellyn (1985): Fine structure of aphid stylet pathways and its use in host plant resistance studies. *Entomol. exp. appl. 38*, 293-295.

Tjallingii, W. F. (1988): Electrical recording of stylet penetration activities. In: Minks, A. K. and P. Harrewijn (eds), *Aphids, Their Biology, Natural Enemies and Control.* Vol. 2B. Elsevier. Amsterdam

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INCREASED INFESTATION OF NORWAY SPRUCE SAPLINGS BY THE APHID *CINARA PILICORNIS* HARTIG AS A CONSEQUENCE OF EXPOSURE TO FLUORIDE, NITROGEN COMPOUNDS AND SO_2

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Key words: air pollution, aphids, sulphur dioxide, nitrogen, fluoride, norway spruce, *Cinara pilicornis, Picea abies.*

In woody plants stressed by air pollutants increased development of aphid populations is reported in several studies (e.g. Dohmen, 1988; Braun and Flückiger, 1989). We report observations of lachnid aphids on spruce saplings exposed to some air pollutants.

Experiments were carried out in a field where five-years-old Norway spruce (*Picea abies* (L.) Karst) saplings were growing. Pollutant deposition on saplings was simulated by spraying NaF (F 30 mgl^{-1}) and nitrogen compounds ($Ca(NO_3)_2$ and $(NH_4)_2SO_4$, N 200 mgl^{-1}) as aqueous solutions and fumigating plants with gaseous SO_2 separately and in mixtures. Control seedlings were sprayed with water. Occurrence of the spruce shoot aphid (*Cinara pilicornis* Hartig) on saplings was monitored in two weeks intervals. In the same plots (not in N+F and N+SO_2 treatments) eight three-year-old spruce seedlings were growing in 1500 ml containers. Four apterous females were placed on each seedling and the aphid numbers were counted weekly.

Free amino acids from stems of latest shoots of the naturally infested saplings in control and F-treatments were analysed in the end of the following growing season. Fluoride content in the needles were analysed with a microdiffusion method (Kari et al., 1976).

In natural infestation exposures to fluoride, SO2 and nitrogen produced 300%, 200% and 100% increases, respectively in the numbers of aphids during the peak populations. The strongest interactive effect was found between nitrogen and SO_2. In artificial infestation significantly increased aphid populations were observed in fluoride treatment and in combination treatments (SO_2+N and SO_2+N+F). In the end of the growing season the concentrations of F in the needles were 5.2, 22.8 and 23.1 μgg^{-1} DW, in control, F and SO_2+N+F treatments, respectively. Our results support the earlier results (Villemant, 1981; Thalenhorst, 1974) that especially fluoride pollution has an aphid growth promoting effect on conifers. There were no significant differences in the concentrations of free amino acids in shoot stems between control and fluoride treatment. However, the relative low concentration of arginine in F treatment at the end of growing season might indicate disturbances

in the nitrogen metabolism of spruce saplings. Normally arginine concentrations are lower during active growth than during the dormancy (Durzan, 1968). Fisher (1987) found this essential amino acid to be less concentrated on spruce foliage uninfested with the aphids than in infested foliage. Arginine may be an indicator of nutritional suitability of host plant for conifer aphids.

REFERENCES

Braun, S. and W. Flückiger (1989): Effect of ambient ozone and acid mist on aphid development. *Environ. Poll. 56*, 177-187.

Dohmen, G. B. (1988): Indirect effects of air pollutants: changes in plant-parasite interactions. *Environ. Poll. 53*, 197-207.

Durzan, D. J. (1968): Nitrogen metabolism of *Picea glauca*. I. Seasonal changes of free amino acids in buds, shoot apices, and leaves, and the metabolism of uniformly labelled ^{14}C-L-arginine by buds during the onset of dormancy. *Can. J. Bot. 46*, 909-919.

Fisher, M. (1987): The effect of previously infested spruce needles on the growth of the green spruce aphid, *Elatobum abietinum*, and the effect of the aphid on the amino acid balance of the host plant. *Ann. Appl. Biol. 111*, 33-41.

Kari, T., P. Kauranen and E. ja Alhava (1976): Effect of drinking water fluoridation on the flouride content of human bone in and around Kuopio, Central Finland. Analytical study. *Publ. Univ. Kuopio, Comm. Health Ser. 1*, 1-15.

Thalenhorst, W. (1974): Untersuchungen über den Einfluss fluorhaltiger Abgase auf die Disposition der Fichte für den Befall durch die Gallenlaus *Sacchiphantes abietis* (L.) *Z. Pflkrankh. Pflschutz 81*, 717-727.

Villemant, C. (1981): Influence de la pollution atmosphérique sur les populations d'aphides du pin sylvestre en forêt de Roumare (Seine-Maritime). *Environ. Poll. (Ser. A) 24*, 245-262.

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A METHOD FOR DEMONSTRATING SAP UPTAKE IN THE RICE BROWN PLANTHOPPER, *NILAPARVATA LUGENS,* USING A RADIOACTIVE TRACER TECHNIQUE

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Key words: *Nilaparvata lugens,* rice brown planthopper, radioisotope, electronic recording, sap ingestion, host plant resistance, honeydew collection.

The feeding patterns of the brown planthopper, *Nilaparvata lugens,* (Hemiptera, Delphacidae) can be monitored by incorporating it into an electrical system, as devised by Tjallingii (1978). Although this technique gives information on the feeding duration, the amount of fluid ingested cannot be deduced. Thus sap uptake was investigated directly by incorporating a radioactive tracer into the rice plant. The roots were immersed into a 1μCi/ml solution of 32 — Phosphorus, prior to the start of the experiment. The insect was allowed to feed for 24 hours on the labelled plant with the feeding patterns being recorded simultaneously. Honeydew droplets were collected and these, as well as the insect, were assessed for radiation.

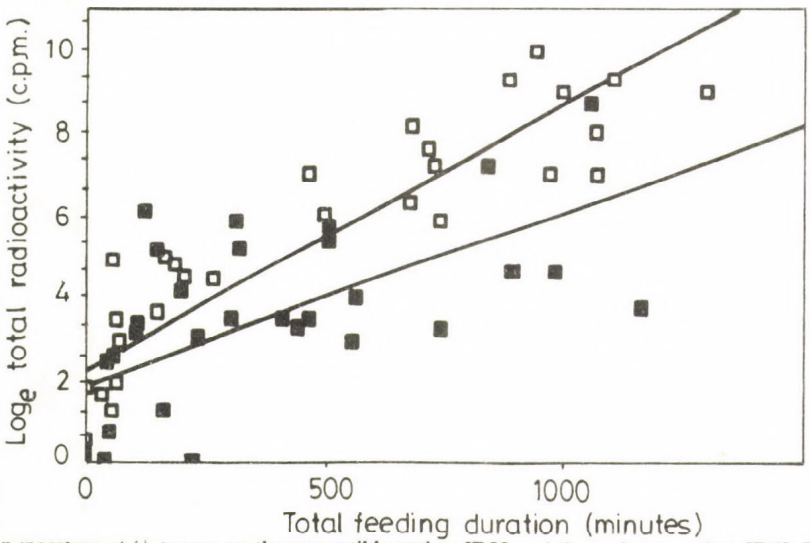

Fig. 1 Sap ingestion of N. lugens on the susceptible variety IR22 and the resistant variety IR62. Total radioactivity of insect and honeydew against total duration of feeding patterns.
□IR 22 – r=0.90; p=<0.001; $y=6.84*10^{-3}x+1.56$
■IR 62 – r=0.67; p=<0.001; $y=4.63*10^{-3}x+1.73$

The results illustrate that insects feeding for sustained periods on the susceptible cultivar IR22, ingest at a greater rate than those insects that feed for short periods.

Also a resistance mechanism has been illustrated in cultivar IR62 which can cause a 60 times reduction in sap ingestion.

REFERENCE

Tjallingii, W. F. (1978): Electronic recording of penetration behaviour by aphids. *Entomol. exp. appl. 24,* 521-530.

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THE EFFECT OF NUTRITION ON OUTBREAKS OF THE AFRICAN ARMYWORM, *SPODOPTERA EXEMPTA* (WALKER)

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Key words: Lepidoptera, *Spodoptera exempta*, nutrition, outbreak, nitrogen, East Africa, Kenya, Gramineae.

The African armyworm, *Spodoptera exempta* (Walker), which feeds almost exclusively on plants of the families Gramineae and Cyperaceae, periodically reaches high population densities (up to 1000 larvae per m^2) in East Africa (Brown, 1962). Crops like maize, sorghum and millet may be devastated and grazing land may be turned bare (Odiyo, 1979).

Founding populations of the African armyworm occur at low to very low densities during the long dry season (July-October). At the beginning of the rainy season (late October-early November) a rapid build-up of the population may occur in the so called primary outbreak areas due to favourable conditions. The adults arising in these primary outbreak areas migrate downwind over large distances and may found new generations in secondary outbreak areas. After two generations a multiplication of 10,000 fold has been observed (Odiyo, 1979, 1981; Tucker, 1984).

It is imperative to control this insect in its primary outbreak areas, which requires a thorough knowledge of the factors which contribute to the timing and successful development of armyworms in these areas.

The hypothesis has been postulated that the upsurge of mineral nitrogen in the soil caused by rain influences, via its effect on the insect's host plants, larval growth and adult performance of the African armyworm.

In 1988 preliminary experimental work has been executed in the field to test the above mentioned hypothesis. Soil and plant (wild grasses) samples have been collected in Kitui District, Kenya, an area where frequently first outbreaks occur during the 'armyworm season'. Three sample areas were chosen: 1) Yatta (1°45' South, 37°82' East, alt. 1040 m), 2) Kanyangi (1°75' South, 37°96' East, alt. 1040 m), and 3) Ikutha (2°04' South, 38°10' East, alt. 700 m).

Weather: The onset of the short rainy season (day 0 in Figure 1) was October 25 for Yatta and November 7 for Kanyangi and Ikutha.

Soil: Immediately after the first rains, decomposition of organic material by bacterial activity increases rapidly. In Kanyangi and Ikutha and to a lesser extent in Yatta, this resulted in a short flush of accessible nitrogen in the soil (Fig. 1A). While

this flush is supposed to be positively correlated with the intensity of the preceding drought (Birch, 1959), it has been observed that more severe drought periods are followed by larger armyworm infestations. This supports the idea of a causal relationship as postulated in the above mentioned hypothesis.

Plant: During the long dry season there is no vegetation which can serve as food for armyworms. Immediately after the first rains, however, there is a flush of young, soft, and highly nutritious grasses and crops which is stimulated by the upsurge of accessible nitrogen and which is ideal food for young armyworm larvae (Fig. 1B).

Armyworm: The phenology of the African armyworm is very well timed (Fig. 1C). Because the moths arrive with the first rains, the caterpillars develop when the plants seem to be most nutritious.

The nutrional quality of the grasses was high during the experimental period, probably as a consequence of the peak in decomposition of organic material. To which extent nutritional quality of the food affects larval growth and adult performance of the African armyworm still has to be investigated.

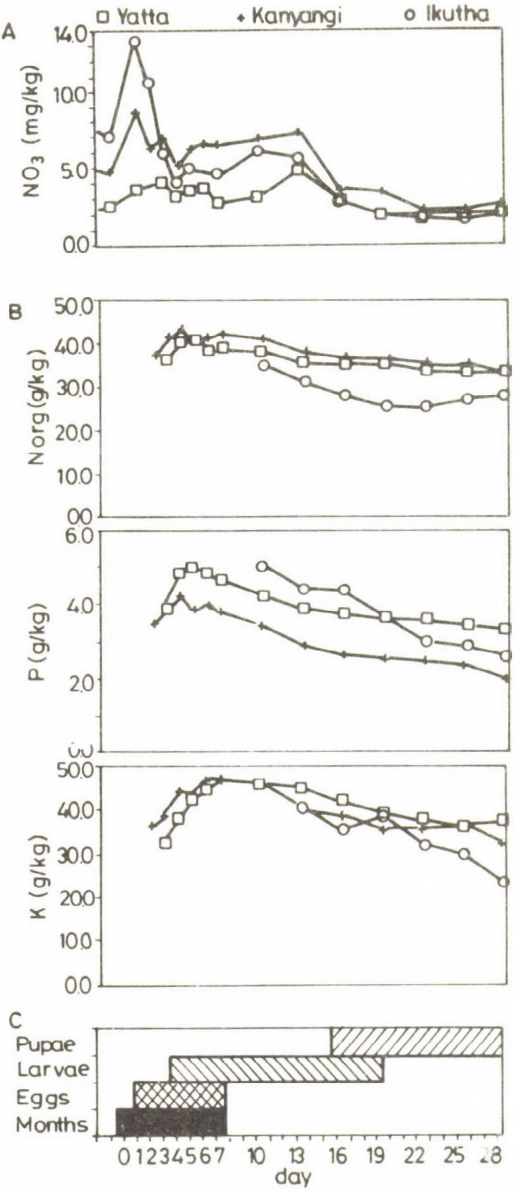

Fig. 1 *A* Nitrate content of the soil just before and during the first month after the onset of the short rainy season in the three sample areas. *B* Organic nitrogen, phosphorus and potassium content of the grasses. *C* Phenology of S. *exempta*.

REFERENCES

Birch, H. F. (1959): Further observations on humus decomposition and nitrification. *Plant and Soil 11*, 262-286.

Brown, E. S. (1962): The African armyworm, *Spodoptera exempta* (Walker) (Lepidoptera, Noctuidae): review of the literature. Commonwealth Inst. Entomol., London.

Odiyo, P. O. (1979). Forecasting of a migrant pest: the African armyworm *Spodoptera exempta* (Walk.). *Phil. Trans. R. Soc. Lond. B 287*, 403-413.

Odiyo, P. O. (1981): Development of the first outbreaks of the African armyworm, *Spodoptera exempta* (Walk.) between Kenya and Tanzania during the 'off season' months of July and December. *Insect Sci. Applic. 1*, 305-318.

Tucker, M. R. (1984). Possible sources of outbreaks of the armyworm, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae), in East Africa at the beginning of the season. *Bull. entomol. Res. 74*, 599-607.

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METABOLIC CONSEQUENCES OF STRESS INDUCED BY THE FEEDING OF *TETRANYCHUS CINNABARINUS* ON TOMATO PLANTS

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Key words: *Tetranychus cinnabarinus*, tomato plants, reducing sugars, soluble protein, total phenols, stress

When exposed to mite feeding, susceptible plants severely suffer from metabolic disfunction and structural injuries (Sances et al., 1979; Mothes and Seitz, 1982; De Angelis et al., 1983; Kielkiewicz, 1985; Tomczyk and Kropczynska, 1985). Resistant (or tolerant) plants' response to mite attack depends on the adjustment of their metabolism and growth (Hildebrand et al., 1986). On the other hand, biochemical changes in hostplants may influence the density of mite population.

In this study I tried to check:

1) the ability of mite-infested plants to mobilize some organic compounds, and
2) the influence of biochemical changes in infested leaves on mite fecundity.

Tomato plants of a mite-susceptible cultivar (Celena) and a partly resistant cultivar (Novy) were used in the experiments. Each plant was infested by 3 adult females of *Tetranychus cinnabarinus* (Boisduval) per leaf. After 4 days and after 3 and 5 weeks some parts of the green leaf including the lesions were sampled and used for biochemical analysis. Eggs laid by *T. cinnabarinus* on leaf disk taken from healthy leaves, 4-day infested leaves and from healthy sites of 4-day infested leaves were counted after 3 days of mite feeding.

First symptoms of *T. cinnabarinus* feeding (yellow lesions with brownish spots but not light punctures typical for other species of spider mites) appeared after 2-4 days of feeding. A progressive increase in lesion size was observed between 3th and 5th weeks of mite infestation. Even a small initial population of *T. cinnabarinus* produced severe damage of leaves and as a result the defoliation of the plants. Defoliation of Celena plants started earlier and was faster than of Novy plants.

Females of *T. cinnabarinus* fed on injured leaves of both cultivars laid 2-3 times less eggs than on uninjured ones. More eggs were found on healthy tissues surrounding injured sites than on injured places.

Biochemical analysis indicated that soon after the infestation period (4 days) the damaged tissues of Novy cultivar contained more reducing sugars, starch, dry material and total phenols than uninjured leaves. A decrease of soluble protein content in infested leaves of Novy plants was observed. The content of starch and total phenols

in damaged leaves of Celena plants insignificantly increased, whereas reducing sugars, soluble protein and dry weight decreased. Prolonged time of mite infestation (3 weeks) resulted in a decrease of starch content and increase of soluble content in leaves of both cultivars. Significant increase of total phenols in leaves of Celena plants was observed. Dry weight of the injured leaves of both cultivars decreased. Five weeks of mite infestation resulted in large decrease of reducing sugars, starch, soluble protein and phenols in infested leaves.

In conclusion, it seems that reaction of infested leaves on mite stress is biphasic: firstly, the mobilization and secondly, the decrease of organic compounds take place. It is possible that differences in sensitivity of tomato plants may depend on time-reaction: plants of the resistant cultivar react faster. It seems also true that the accumulation of different compounds in infestation sites and in the surrounding tissues may be the reason of differences in mite fecundity.

REFERENCE

De Angelis, J., A. B. Marin, R. E. Berry and G. Krantz (1983): Effects of spider mite (Acari: Tetranychidae) injury on essential oil metabolism in peppermint. *Environ. Entomol. 12,* 522-527.

Hildebrand, D. F., J. G. Rodriquez, G. C. Brown and C. S. Volden (1986): Two-spotted spider mite (Acari: Tetranychidae) infestations on soybeans: effect on composition and growth of susceptible and resistant cultivars. *J. Econ. Entomol. 79,* 915-921.

Kielkiewicz, M. (1985): Ultrastructural changes in strawberry leaves infested by two-spotted spider mites. *Entomol. exp. appl. 37,* 49-54.

Mothes, U. and K. A. Seitz (1982): Fine ultrastructural alterations of bean plant leaves by feeding injury of *Tetranychus urticae* Koch (Acari: Tetranychidae). *Acarologia, 23,* 149-157.

Sances, F. V., J. A. Wyman and J. P. Ting (1979): Morphological responses of strawberry leaves to infestations of two-spotted spider mite. *J. Econ. Entomol. 72,* 710-713.

Tomczyk, A. and D. Kropczynska (1985): Effects on the host plant. In: W. Helle and M. W. Sabelis (eds). *Spider Mites. Their Biology, Natural Enemies and Control.* Vol. 1A, Elsevier, Amsterdam. 317-327.

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YPONOMEUTA-SPECIES AND THEIR HOST PLANTS

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Key words: *Yponomeuta*, monophagous, oligophagous, larval food restriction, host plant exchange, seasonal variations

Host plant shift is thought to be a driving force in the evolution of the small ermine moths (*Yponomeuta* Latr.). The ancestors may have dwelled upon the Celastraceae. Six West European species have been subject of study. The larvae of these insects are restricted in food acceptance. *Y. evonymellus* is found on *Prunus padus, Y. cagnagellus* on *Euonymus europaeus, Y. malinellus* on *Malus domestica, Y. padellus* on *Crataegus monogyna* and *P. spinosa, Y. rorellus* on *Salix* spec. and *Y. vigintipunctatus* on *Sedum telephium*.

Under laboratory conditions the species are less restricted in food acceptance than in the field (Fig. 1). Larvae of most species accept food plants belonging to the same genus as their host. Some species even are able to complete larval development on plants belonging to other families, viz. *Y. cagnagellus* on *P. padus, Y. padellus* on *Salix alba* and *Y. rorellus* on *Crataegus monogyna*.

The exchange of hosts is tested (Fig. 2). The response varied from total refusal to full acceptance. Hosts of *Y. evonymellus* and *Y. padellus* are interchangeable.

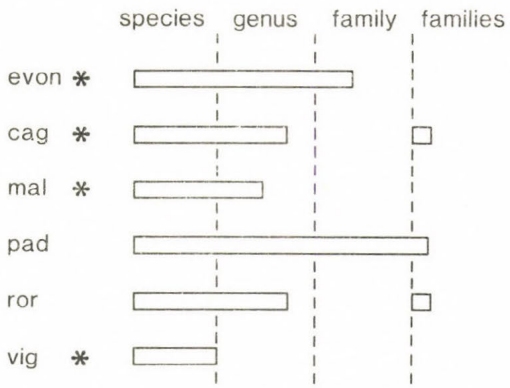

Fig. 1 Larval food restriction. The length of each bar represents the degree of food acceptance. * is monophagous in the field.

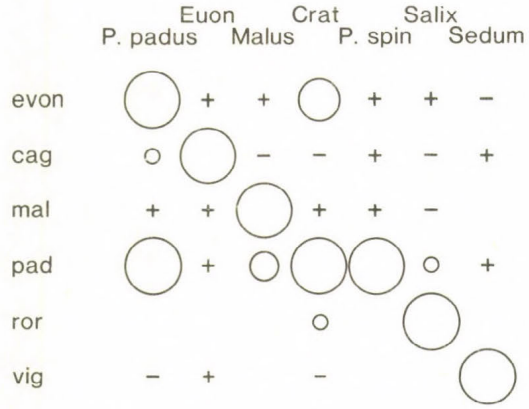

Fig. 2 Acceptance of host plants of *Yponomeuta*-species by second or third instar larvae of these insects. - = no feeding traces; + = feeding traces; size of circle indicates success (survival + pupal weight). Empty spaces are missing observations.

Food acceptance is influenced by seasonal variations in plant quality (Fig. 3). It appears that the two monophagous species are less flexible in this respect than the oligophagous one.

On a phylogenetic tree (Herrebout, 1990) species that accept each other's host are found close together (Fig. 4). *Y. padellus, Y. rorellus, Y. evonymellus* and *Y.*

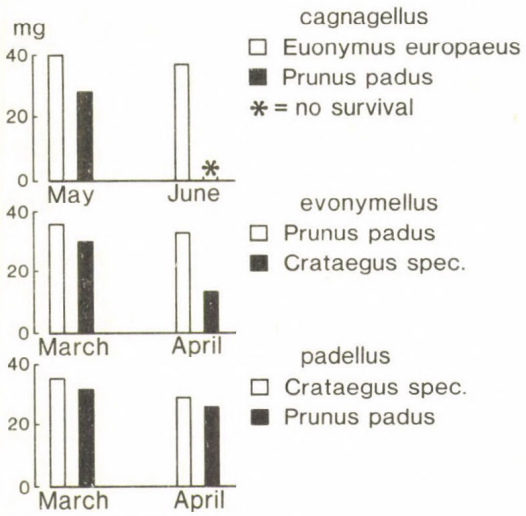

Fig. 3 Seasonal influence of food plant quality on pupal weight. Host plant = white and non-host plant = black.

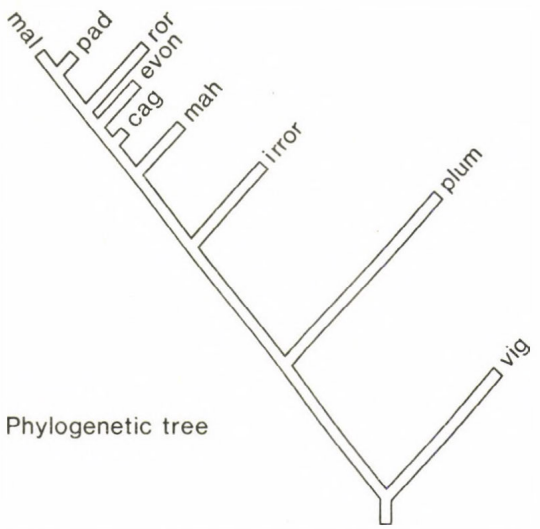

Fig. 4 mal = *malinellus*; pad = *padellus*; ror = *rorellus*; evon = *evonymellus*; cag = *cagnagellus*; mah = *mahalebellus*; irror = *irrorrellus*; plum = *plumbellus*; vig = *vigintipunctatus*.

cagnagellus can develop on hosts of some of the neighbouring species. *Y. malinellus*, however, cannot cope with the host of its most close relative.

ACKNOWLEDGEMENTS

I am grateful to Mssrs. H. Heijn and M. L. Brittijn for preparing the figures.

REFERENCE

Herrebout, W. M. (1990): Phylogeny and host plant specialization: small ermine moths (*Yponomeuta*) as an example. This volume.

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TRENDS IN THE SPECIATION OF SOME HOMOPTERA GROUPS IN ASSOCIATION WITH HOST PLANTS

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Key words: Coccoidea, Diaspididae, Aleyrodoidea, scale insects, aphids, sequential evolution, stabilizing selection, isolate selection, monophagous, polyphagous, woody and herbaceous plants, Palearctic Region, Central Europe, apparent.

The ways of the speciation in different groups of phytophagous insects are not very clear. There are great differences in the intensity of speciation in various groups of insects (aphids, scale-insects, etc.) and in different geographical regions. The study of these questions might be able to give new insights for the further development of Jermy's (1984) theory of sequential evolution.

I studied the number of plant species and Homoptera species, more specifically the Aleyrodoidea and Coccoidea on a world and on regional scales, including the questions of rarity and the rate of speciation.

It has to be stressed that more than 20% of species of some homopteran groups have been described after 1960, what shows that our taxonomic knowledge is still rather incomplete, and, therefore, generalizations may be premature.

According to the available data there is no correlation between the number of plant species (232.000) and that of the homopterous species (13.000) on a world scale. This indicates that the species richness of the plant kingdom as a whole plays a very restricted role in the evolution and speciation of homopterous insects.

One important way of speciation in these insects might have been "isolate selection" (Glazier, 1987), which could result in a high ratio of monophagous and oligophagous species among all homopterous groups (74.1% in the case of Diaspididae and 75.3 in Aleyrodoidea on a world scale). This process seems to work mostly on less apparent herbaceous plant species, and especially on perennial ones. In case of scale insects of the Palearctic Region, plant genera such as *Agropyron, Festuca, Stipa, Carex, Thymus, Dianthus, Artemisia, Calluna, Erica, Luzula,* etc. belong to this group. The 87% of Diaspididae species of the world show very restricted distribution, and almost all newly described species are rare and monophagous. Most of the scale insects in Central Europe are known primarily on relatively rare, perennial herbaceous plants and secondly on rare woody plants (Fig. 1). This agrees with Kovalev's (1990) assumption that after the destruction of the climax communities by ecological crises in the Oligocene and Miocene, there appeared new dominant plants, like the above, that provided a good opportunity for the radiation processes.

Another important way of speciation for scale insects is "stabilizing selection" (Smalhausen, 1968). The widely distributed scale insect species on the highly apparent woody plants (*Rosa, Tilia, Betula, Acer, Prunus, Malus, Fagus, Juglans, Populus, Salix,* etc.) are polyphagous and, there is no clear trend of speciation. On fruit trees, 83% of the world scale insect species are polyphagous (Fig. 2). According to Kovalev (1990), the polyphagous species occupy the early stages of succession on phylogenetically young taxa, however, while the scale insects remained polyphagous, the aphids became highly specialized on these plants that could be explained by the different rates of evolutionary processes in this two groups of insects.

Only a small grop of scale insects shows a clear radiation on some widely distributed plants (*Quercus, Pinus, Picea*, etc.), which could be explained by the very long geological apparency (age) of these plants.

I conclude that the speciation in these groups of insects is determined mostly by historical events, ecological crises (glaciation), geographical and other factors of

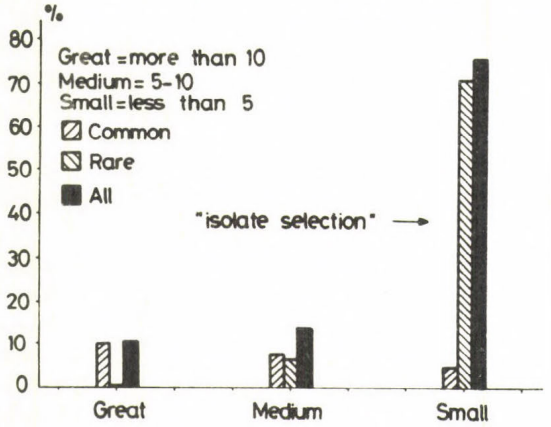

Fig. 1 Scale insect species reachness on common and rare plants in Central Europe.

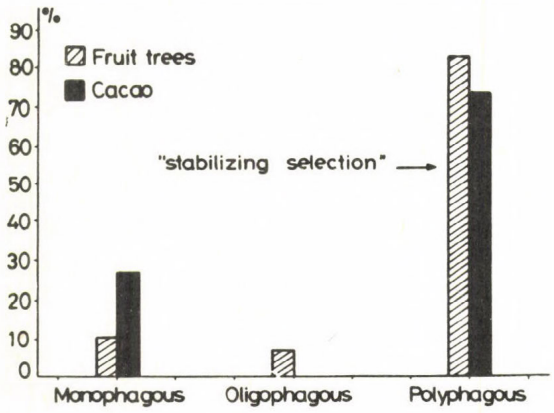

Fig. 2 Host specialization of *Diaspididae* species on woody plants in the World.

isolation (isolate selection processes) and by the genetic possibilities of these insects (species radiation and stabilizing selection processes). All of these show that ecological methods alone are often unsuitable for studying the evolutionary processes.

REFERENCES

Glazier, D. S. (1987): Toward a predictive theory of speciation: The ecology of isolate selection. *J. theor. Biol. 126,* 323-333.

Jermy, T. (1984): Evolution of insect/host plant relationships. *Am. Nat. 124,* 609-630.

Kovalev, O. V. (1990): The role of evolution of the dominant species of succession processes in the Cenozoic ecosystems in the formation of oligophagy of recent phytophages. This volume.

Smalhausen, I. I. (1968): *Cybernetic Questions of Biology*. Nauka, Novosibirsk. (In Russian)

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ECOLOGY OF MITE COMMUNITIES OCCURRING ON ORNAMENTAL TREES IN TOWNS

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Key words: *Tilia cordata, Quercus robur, Eotetranychus tiliarum, Oligonychus querci,* ornamental trees, urban habitats, predatory mites, mite communities.

The data presented in this paper are a continuation of the studies on the ecology of mite communities in urban habitats. Previous studies were limited to the mite species occurring on linden. Results were discussed by Kropczynska (1984), Kropczynska et al. (1985, 1988). In 1986-88 a comparative study of mite communities occurring on linden and oak was carried out.

Two types of habitats: urban street trees and park trees were studied to determine the species composition, structure of communities and seasonal dynamics of species concerned. 20-30-year-old *Tilia cordata* Mill. and *Quercus robur* L. growing at 3 localities (five trees at each locality) along main streets and 3 in the park were selected for experiment. Samples of 100 leaves per tree were collected every 14 days and all mites on the leaves were counted and identified.

To collect data on the bionomics of *Oligonychus querci* sp. n., tetranychid mites occurring on oak, laboratory experiments were conducted according to the method described for *Eotetranychus tiliarium* (Kropczynska, 1984). On the basis of the data obtained a life table was constructed. It was found that the mite community on linden and oak at the urban habitat consisted primarily of phytophagous and predacious species. No evident differences were found in the species composition of the communities inhabiting park and street trees. High densities of spider mites occurred only on linden trees growing along streets. On oak this phenomenon was not observed. High reproductive rate (R_o) of *E. tiliarium* can be one of the reasons of these outbreaks.

On linden the population of predatory mites responded numerically to the increase of the number of spider mites. On oak the average density of predatory mites oscillated in the range 0.1 to 1 mite per leaf. Increase in density of Tetranychidae did not influence the numbers of predators.

REFERENCES

Kropczynska, D. (1984): *The role of predacious mites (Phytoseiidae) as natural enemies of* Eotetranychus tiliarium *(Hermann) in town conditions.* Treatises monographs Warsaw Agric. Univ. (In Polish).

Kropczynska, D., M. van de Vrie and A. Tomczyk (1985): Woody ornamentals in World crop pests. In: Helle, W. and M. Sabelis (eds), *Spider mites. Their Biology, Natural Enemies and Control*, Vol. 1B. Elsevier, Amsterdam. 385-393.

Kropczynska, D., M. van de Vrie and A. Tomczyk (1988): Bionomics of *Eotetranychus tiliarium* and its phytoseiid predators. *Exp. Appl. Acarology, 5,* 65-81.

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APPLYING OF ARTIFICAL DIET IN THE REARING OF COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA* SAY) ADULTS

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Key words: 17α-methyl-17β-hydroxyandrostadien-1,4-on-3, chlorogenic acid, phospholipids, phagostimulants, metanabol, survival.

A basic diet (BD) for the Colorado potato beetle was developed (Krzymańska and Zwolinska, 1970) which enabled to rear adult beetles and examine the influence of plant material as well as synthetic compounds for feeding and development of the insect. As plant material dried potato leaves from different parts of plant and in different doses were tested. As phagostimulants plant phospholipids, chlorogenic acid and metanabol (17α-methyl-17β- hydroxyandrostadien-1,4-on-3) were applied. Percentage of feeding insects, survival, weight gain and lipid content after 30 days of rearing, were recorded.

Feeding response of beetles reached a high value in all cases when dried potato leaves were added, and also on the diet containing plant phospolipids. The survival of beetles, weight gain and content of lipids were the highest on diets supplemented with potato leaves together with metanabol or chlorogenic acid.

The results showed the very positive effect of dried potato leaves, especially top leaves. It is indicated that host-plant material contains specific stimulants, necessary for a proper development of the beetles. The addition of chlorogenic acid and metanabol showed a positive effect on the beetles' development, as it was also stated in larval development. Plant phospholipids had no positive effect on insect develop-

Table 1 The effect of phagostimulants on Colorado potato beetles reared on artificial diet

Diet	Feeding %	Survival %-30 day	Final weight in mg	Content lipid % fr.w.
Basic diet (BD)	57.5	58.8	108	1.40
BD + 5 g top leaves	100	69.2	119	4.30
BD + 10 g top leaves	100	76.9	132	6.38
BD + 20 g top leaves	100	80.0	138	8.84
BD + 20 g bottom leaves	100	56.0	111	5.25
BD + 20 g top leaves + chlorogenic acid	100	82.6	143	8.34
BD + 20 g top leaves + metanabol	100	86.7	146	8.62
BD + plant phospholipids	100	16.7	79	1.20

ment, in spite of the fact, that these compounds had positive effect on feeding response.

REFERENCE

Krzymańska, J. and Z. Zwolińska-Śniatałowa (1970): Elaboration of basic artificial diet composition for the Colorado potato beetle. *Biol. Acad. Pol. Sci. 18*, 785-787.

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PHENOLIC COMPOUNDS IN *BRASSICA* AS RESISTANCE FACTORS AGAINST *PIERIS* CATERPILLARS

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Key words: phenolic acids, flavonoids, host plant resistance, *Pieris brassicae*, *Pieris rapae*, *Brassica oleracea*.

The caterpillars of *Pieris brassicae* and *P. rapae* (Lepidoptera: Pieridae) are crucifer specialists. Cruciferous plants contain glucosinolates, compounds thought to constitute a chemical defence barrier against herbivorous attack. Apparently, this barrier is not effective against *Pieris* caterpillars. Apart from glucosinolates, members of the genus *Brassica* also contain phenolic acids and flavonoids. Several phenolic compounds inhibited larval survival, growth and development when *Pieris brassicae* and *P. rapae* larvae were continuously exposed to these compounds by presenting them in an artificial diet (van Loon, 1988). The dietary concentrations at which these effects occurred are in the range of reported levels of identical or related compounds naturally occurring in *B. oleracea* L. The objectives of this study were to investigate larval performance of both *Pieris* species on different cultivars of *B. oleracea*, including four with reported resistance to *P. rapae*, and to analyse leaf tissues of these host species to test the hypothesis that phenolic metabolism of *B. oleracea* contributes to defence against *Pieris* caterpillars.

Survival, development, growth from egg to pupa and pupal weights were used as parameters to assess the performance of both species on seven cultivars. Survival and development were monitored every 48-60 h during the first 12 days after egg hatch. Excised leaf material was used. The concentrations of the main aglycones of phenolic acids and flavonoids were determined in the leaves of 5 of the cultivars by high pressure liquid chromatography (HPLC). In both caterpillar species, significant differences in most of the performance parameters were found between the cultivars. Each of the cultivars had its own quantitative pattern of phenolic acids and flavonoids. In all cultivars analyzed, 4 phenolic acids were prominent. The separation of flavonoids was found to be improved by the use of tetrahydrofuran as an eluent. This modification revealed the presence of at least 5 unidentified components in the HPLC-chromatogram in addition to quercetin and kaempherol, both common flavonoids in Cruciferae. It is not well possible to integrate the results of the artificial diet study with the differential suitability of the host plants. The host plants most probably differed in several other characters than their widely varying levels of phenolic acids and flavonoids alone. The effects of mixtures of compounds have not been tested in the artificial diet study. Nevertheless, the combined results suggest

that phenolic acids and flavonoids in *B. oleracea* may be involved in defence against *Pieris*.

REFERENCE

Loon, J. J. A. van (1988): *Sensory and nutritional effects of amino acids and phenolic plant compounds on the caterpillars of two Pieris species.* Thesis, Agricultural University, Wageningen.

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IMPORTANCE OF QUINOLIZIDINE ALKALOIDS TO THE SPECIALIST HERBIVORE *URESIPHITA REVERSALIS* (LEPIDOPTERA: PYRALIDAE)

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Key words: *Uresiphita reversalis*, aposematic, alkaloid, Lepidoptera, Pyralidae, legume, sequester, defense, predation, cuticle, sparteine, cytisine, phagostimulant.

Larvae of *Uresiphita reversalis* feed almost exclusively on legumes in the tribe Genisteae, which characteristically contain a variety of quinolizidine alkaloids. The larvae are aposematic, and on French broom, *Cytisus monspessulanus*, they feed on the youngest leaves, at the periphery of the plant. These leaves, which were preferred over older foliage in choice tests, contained four times the level of alkaloids found in older leaves.

Quinolizidine alkaloids that occur in host plants were found to be phagostimulatory. When larvae were given a choice between sucrose-impregnated glass fiber disks with or without alkaloid (cytisine or sparteine) added they preferred cytisine-treated disks to control disks at all concentrations between 0.1 and 2.0% dry weight ($p<0.01$, Sign test). They ate more of sparteine-treated disks than control disks, though the differences were only significant when all the data were pooled ($n=61$, $p<0.05$, Sign test).

There appears to be a long-term benefit of moderate sparteine levels on growth of larvae of *U. reversalis*. This was tested by adding alkaloids to host plants relatively low in alkaloids of their own. A pair of branches was chosen on potted plants of *C. monspessulanus*. Branches were dipped in alkaloid (sparteine or cytisine in 70% ethanol, made to a concentration which added approximately 0.5% dw alkaloid to foliage), or in ethanol alone. Newly molted last instar larvae were bagged on these branches until pupation. Pupal weights of insects fed sparteine-treated foliage were significantly higher than those fed untreated foliage (119.1 vs. 113.0 mg; $p<0.05$, Wilcoxon's signed-ranks test). Relative growth rates were .048 and .045 on sparteine treated and control foliage ($p<0.05$, Wilcoxon's signed-ranks test). There were no significant differences in the developmental time or in the relative egestion rate (mg frass/mg larva/day) for insects on alkaloid-treated plants. Cytisine treatment had no significant effect on any of the growth parameters measured.

Ingested alkaloids are retained to some extent by larvae of *U. reversalis*. A highly significant correlation exists between the level of alkaloids in broom plants and larvae ($r=0.91$, $p<0.001$), and between levels in plants and frass ($r=0.96$, $p<0.01$). Over

98% of the plant alkaloids retained by the larvae were recovered from the exuviae of the last larval instar. None were found in pupae. This suggested a protective role for the alkaloids in these aposematic larvae.

An extract of the exuviae of last instar *U. reversalis* and a surface extract of freshly killed *U. reversalis* applied to normally palatable potato tuber moth larvae both significantly deterred ants, *Iridomyrmex humilis*, compared to solvent-treated controls (number of trials with trails of foraging ants=11-15, $p<0.025$, <005, Wilcoxon's signed-ranks test). Both extracts contained plant-derived quinolizidine alkaloids. An extract of larval exuviae was significantly more deterrent to ants than an extract of pupal exuviae. Further work is in progress on the functional value of these alkaloids to *U. reversalis* in relation to defense against predators.

ACKNOWLEDGEMENT

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THE ROLE OF HYDROXAMIC ACIDS IN THE RESISTANCE OF MAIZE TO INSECTS

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Key words: DIMBOA, phytochemicals, maize, *Rhopalosiphum padi*, damage stress, drought stress.

The 4-hydroxy-1,4-benzoxazin-3-one group of hydroxamic acids (Hx) are present in many Gramineae. They are known to confer resistance to insects such as aphids (Bohidar et al., 1986) and Lepidoptera. In maize, the predominant Hx is DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), and is normally present in the plant tissue as the glucoside which is enzymically hydrolysed upon damage.

The aim of the research was to examine the effect of stress factors, such as damage and drought, on the Hx levels in maize, and to determine if this is related to insect growth and survival.

The analysis of Hx in maize (cultivar LG11, Elsoms Seeds, U.K.) was carried out using the ferric chloride technique described in Bohidar et al. (1986), while Hx in aphids was determined using high performance liquid chromatography (Niemeyer et al., 1989). Most of the experiments were conducted in a glasshouse at approximately 20 degrees centigrade.

In maize, the concentration of Hx in each leaf showed a logarithmic decline with time, with newly-emerging leaves generally having the highest concentrations. Crushing a maize leaf caused a significant increase ($F=4.759$; d.f.$=1,96$; $P<0.05$) in Hx concentration within the whole plant (Fig. 1). Most of the increase was in the damaged and the newly emerging leaves.

The concentration of Hx in drought-stressed plants increased ($F=12.612$; d.f.$=1,108$; $P<0.001$) compared with that in controls. This was due largely to an increase in total Hx. However, abcissic acid, a plant hormone which is produced under drought stress, was found to reduce significantly ($F=17.404$; d.f. $= 1,14$; $P<0.01$) the Hx concentration of isolated maize leaves, while an auxin (indole acetic acid) solution had no apparent effect.

Aphids (*Rhopalosiphum padi* L.) caged on maize leaves partly damaged by crushing, had significantly higher mortality (G-statistic, with Williamsons' correction = 9.3079; d.f.$=1$; $P<0.005$) than those caged on undamaged controls. Aphids caged on maize leaves which had previously been infested with aphids also showed a significantly lower relative growth rate ($F=4.627$; d.f.$=1,32$; $P<0.05$) in the first 24 hours

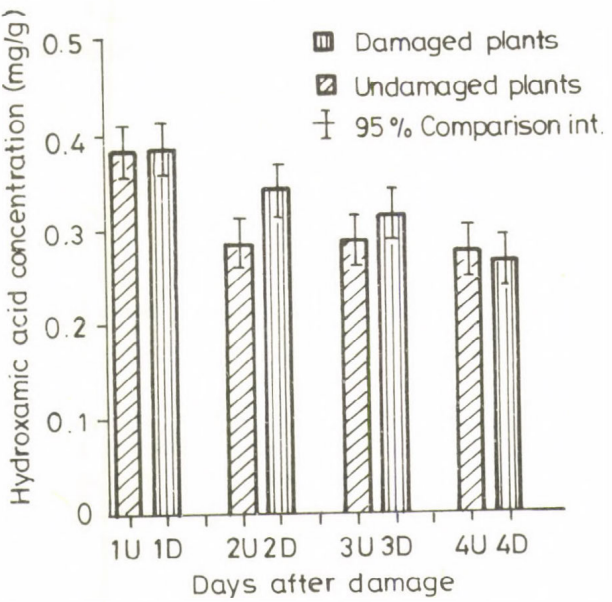

Fig. 1 Hx concentration and 95% comparison intervals for undamaged (U) and damaged (D) maize plants.

compared with controls. The mechanism involved in these responses is not known, but Hx was found in the bodies of aphids feeding on maize.

In future work, a range of maize cultivars will be tested for their Hx content, and attempts will be made to determine if there is a link between this variable and the performance of sucking and leaf feeding insects. Phloem sap will also be examined for the presence of Hx.

REFERENCES

Bohidar, K., S. D. Wratten and H. M. Niemeyer (1986): Effects of hydroxamic acids on the resistance of wheat to the aphid *Sitobion avenae*. *Ann. Appl. Biol. 109*, 193-198.

Niemeyer, H. M., E. Pesel, S. V. Copaja, H. R. Bravo, S. Franke and W. Francke (1989): Changes in hydroxamic acid levels of wheat plants induced by aphid feeding. *Phytochemistry 28*, 447-449.

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A STUDY ON THE CHOICE AND COMSUMPTION OF FOOD BY *ATHALIA ROSAE* L. (HYM., TENTHREDINIDAE)

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Key words: *Athalia rosae*, consumption index, growth rate, food utilization

The purpose of our study was to reveal the appearant differences in food specializations of *Athalia rosae* L. in relation to the main and secondary food plants. Furthermore our task was to determine the value of different food plants from the point of view of ontogeny with the help of the feeding index and the feeding physiological indices (CI, GR, ECI).

The experiments were done in the Institute of Plant Protection of the University of Agriculture in Keszthely. The used larvae originated from a laboratory colony. The larvae were collected in autumn from rape fields and were reared at longday 17/7 LD photoperiod and 22 °C, with the method described by Sáringer (1957).

The following food plants were used: rape, white mustard, savoy, kohlrabi, cauliflower, broccoli. The investigations were adjusted at four different times during the year (June 2, July 8, September 8, November 9). Rape was regarded as the control plant. We put the larvae one by one into the higrostate and after 24 hour feeding the quantity of consumed leaves and the change of weight of the larvae were determined. The investigations were carried out at 15-25 repetitions. The evaluation was done with the method of Waldbauer (1964) and Sáringer (1967).

From the data the consumption index (CI), the relative growth rate (GR) and the brutto efficiency, also the efficiency of the transformation of the ingested food into body mass (ECI) was calculated (Waldbauer, 1964).

The plants belonging to the family of Cruciferae were readily consumed by the larvae of the rape wasp. Savoy (CI = 1.0558), kohlrabi (CI = 0.8021), and rape (CI = 0.8368) plants were eaten on the largest scale, broccoli was consumed in smaller quantity (CI = 0.7904), but we are on the opinion that this latter plant was also a main foodplant of the rape wasp.

The utilization of the plants tested was different. The larvae utilized at best the mustard (ECI = 199.36) and the kohlrabi (ECI = 0.9992) the least. The efficiency of rape can be said as medium.

The growth of larvae is not determined by the quantity of consumed plants, but by the scale of their utilization. Between the relative growth rate (GR) and the brutto

efficiency is a correlation, the individual plants had an effect on the growth of larvae adequate of their value of efficiency.

REFERENCES

Sáringer, Gy. (1957): Die Rübenblattwespe *Athalia rosae* L. (=*colibri*) Christ.) (Tethredinidae, Hym.). *Ann. Inst. Prot. Plant. Hung. 7,* 125-183.

Sáringer, Gy. (1967): Nutrient consumption of the alfalfa weevil (*Hypera (Phytonomus) variabilis* Hrbst.) (Coleoptera, Curculionidae). *Act. Agronomica Hung. 16,* 113-120.

Waldbauer, G. P. (1964): The consumption, digestion and utilization of solanaceous and non-solanaceous plants by larvae of the tobacco hornworm, *Protoparce sexta* (Johan.) (Lepidoptera: Sphingidae). *Entomol. exp. appl. 7,* 253-269.

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FEEDING EFFECT OF LEPIDOPTERA-LARVAE ON THE ARCHITECTURE OF SESSILE OAK (*QUERCUS PETRAEA* MATT. (LIEBL.))

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Key words: Lepidoptera-larvae, sessile oak, defoliation, litter production, branch system pattern, bifurcation angles.

The development of branch system pattern of sessile oak is described by the Rauh's model (Hallé et al., 1978). Originally, in the undisturbed canopy layer of sessile oak the bifurcation angles of new shoots are about 30-60°. As a consequence of the feeding effect at the outbreak of the most frequent Lepidoptera-larvae (Tortricidae, Geometridae, Noctuidae), many new shoots die and shed into the litter (Fig. 1). I found a correlation between the quantity of the damaged current-year shoots falling into the litter and the number of larvae (Nagy, 1981).

In a turkey-sessile oak forest (Síkfőkút Project, near Eger) the annual branch litter production of sessile oak was in 1986 and 1987 an average of 221.07 kg.ha^{-1} (814,500 pieces.ha^{-1}.yr^{-1}). The majority of shed shoots were 1-2 years old (Table 1). The shed shoots falling from one tree averaged in 2,627 pc.ha^{-1}.yr^{-1}, and the lost cumulative leaf area surface was 1 ha.ha^{-1}. After defoliation, when new shoots started to develop in compensation for the reduced leaf area, the original bifurcation angles might have

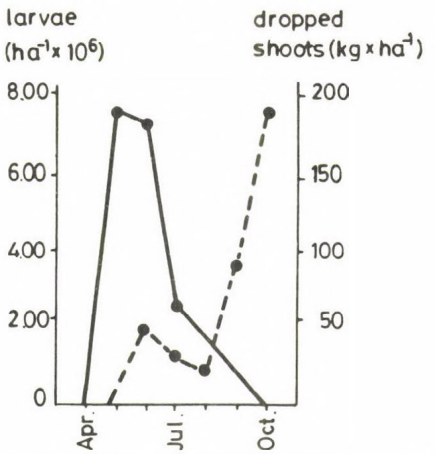

Fig. 1 Distribution of the number of lepidopterous larvae (unbroken line), and the quantity of damaged current-year shoots fallen (broken line) at the sampling area of Síkfőkút Project in 1978.

Table 1 Number of shed sessile oak shoots in litter on the sampling area of Síkfőkút Project in 1987

Period	Shed sessile oak shoot in litter (pieces/ha)				
	Current year	1-year old	2-year old	Older than 2-yr	Total
Dec. 1, '86 – April 30, '87	0	121.000	44.000	20.000	185.000
May 1, '87 – June 30, '87	110.000	120.000	55.000	57.000	342.000
July 1, '87 – Aug. 31, '87	67.000	32.000	23.000	18.000	140.000
Sept 1, '87 – Nov. 30, '87	79.000	36.000	27.000	35.000	177.000
Total	256.000	309.000	149.000	130.000	844.000

changed, becoming often 90-120°. Although the shedding of shoots is continuous throughout the year, a well-defined maximum of litter production could be shown from current-year shoots in May and in June. More than a third (38.2 %) of shed current-year shoots fall into the litter in these two months, and it could be proven that they were largely chewed by caterpillars. Therefore, there is considerable change in number, site, branching angle and leaf rosette pattern of shoots (Fig. 2).

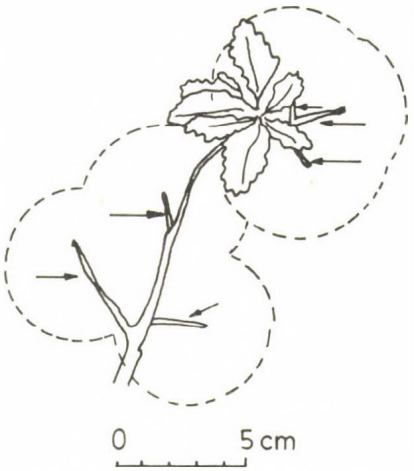

Fig. 2 Feeding effect of lepidopterous larvae on the leaf mosaic within a vegetation period. Arrows indicate the chewed shoots, which will be shed. Dotted circles show the previous leaf rosettes (based on a photo).

REFERENCES

Hallé, F., R. A. A. Oldeman and P. B. Tomlinson (1978): *Tropical Trees and Forests. An Architectural Analysis*. Springer-Verlag, Berlin.

Nagy, M. (1981): The effect of Lepidoptera-larvae consumption on the leaf production of *Quercus petraea* (Matt.) Liebl. *Acta Bot. Acad. Sci. Hung. 27,* 141-150.

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VARIABILITY IN BIRCH AND GYPSY MOTH RESPONSE TO WATER DEFICIT

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Key words: *Betula papyrifera*, *Lymantria dispar*, water deficit, water stress, clones, wilting, feeding, sucrose, larval performance, relative growth rate.

Drought is commonly thought to be associated with insect outbreaks (Mattson and Haack, 1987). We used four clones of paper birch (*Betula papyrifera*) and fourth instar gypsy moth (*Lymantria dispar*) under controlled conditions to investigate the impact of water deficit on insect/plant relationships.

Tissue cultured birches from four geographical locations in North America (S-5, PA-231, WM-148, PA-247) were grown in pots under greenhouse conditions. Water deficit was established by withholding water until wilting at midday was first observed (= day 6). When wilting first occurred, stomatal resistance and photosynthetic rate were measured with a LI-6200, a portable system that uses attached leaves. Leaf samples were taken for later analyses. At that time (day 6), newly molted fourth instar gypsy moth were weighed and then placed on plants. At the end of the stadium, larvae were reweighed, and relative growth rate was calculated. The same procedures were repeated with a second cohort of fourth instars that were placed on plants 10 days after wilting first occurred (= day 16). Leaf sucrose was measured according to procedures developed by Long and Chism (1987).

Water was withheld from plants assigned to stress treatment on day 0. Wilting was first observed on day 6, when larvae were placed on plants. Growth rate of fourth instars increased in response to water stress in two of four birch clones (S-5 and PA-231). On S-5, improved insect performance was detected immediately after stress establishment, then disappeared during feeding by a second larval cohort. On PA-231, larval performance was improved immediately after stress establishment and persisted during feeding by a second cohort. Water stress did not alter host suitability of the other two clones evaluated. There was no correlation between leaf sucrose level and larval performance. Sucrose increased, decreased, or was unchanged in response to water stress. In the two clones that exhibited a change in suitability, sucrose was higher in response to water stress in clone S-5 but lower in PA-231, yet both became better hosts.

Table 1 Relative growth rate of gypsy moth larvae on water-stressed and unstressed clonal birches under greenhouse conditions.

First larval cohort (placed on day 6, immediately after plants wilted)								
Clone	S-5		PA-231		WM-148		PA-247	
	U	S	U	S	U	S	U	S
males	0.158a	0.182b	0.158a	0.170a	0.221a	0.198a	0.170a	0.173a
females	0.171a	0.212b	0.180a	0.202b	0.251a	0l.231a	0.173a	0.181a
Second larval cohort (placed on day 16, 10 days after plants first wilted)								
males	0.183a	0.167a	0.145a	0.158b	0.198a	0.201a	0.152	missing

U=unstressed
S=water stressed
$p<0.02$

REFERENCES

Long, A. R. and G. W. Chism III (1987): A rapid direct extraction-derivatization method for determining sugars in fruit tissue. *J. Food Sci. 52,* 150-154.

Mattson, W. J. and R. A. Haack (1987): The role of drought in outbreaks of plant-eating insects. *BioScience 37,* 110-118.

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THE ROLE OF HYDROXAMIC ACIDS FROM WHEAT IN PEST AND DISEASE RESISTANCE

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Key words: Hydroxamic acids, DIMBOA, Gramineae, wheat, cereal aphids, *Rhopalosiphum padi*, barley yellow dwarf virus.

Hyroxamic acids (Hx) are involved in the defense of cereals against pests and diseases (Niemeyer, 1988). 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), the main Hx in wheat, decreased mean ingestion times by aphids when incorported into artificial diets (Argandoña et al., 1983). Additionally, extracts of aphids contained less DIMBOA when they fed on wheat seedlings with higher Hx levels (Niemeyer et al., 1989).

These results suggest a feeding deterrent effect of DIMBOA towards aphids, which could be valuable in avoidance of barley yellow dwarf virus (BYDV) transmission by their specific aphid vectors.

Choice experiments were carried out with *Rhopalosiphum padi* and paired wheat cultivars varying in Hx levels. When the same cultivars or cultivars with similar Hx levels were used, aphid distribution did not differ significantly from an even distribution. When cultivars with different Hx levels were used aphids were found concentrated in the seedlings with lover Hx levels.

Aphid feeding behaviour on wheat seedlings with varying Hx levels was monitored through electropenetration graphs. In cultivars with higher Hx levels fewer aphids reached the phloem, the time to reach a sieve element was longer and the duration of xylem ingestion was higher.

A survey of 16 cultivars of *Triticum aestivum* was carried out for Hx levels and for reaction to BYDV infection, measured as weight of an hectoliter of grain produced by wheat artificially infested with viruliferous aphids relative to non-infested wheat. While susceptible cultivars were grouped in the low Hx level end of the scale, tolerant ones fell generally in the high Hx level end.

These experiments show the importance of Hx in the acquisition of BYDV by wheat.

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REFERENCES

Argandoña, V. H., L. J. Corcuera, H. M. Niemeyer and B. C. Campbell (1983): Toxicity and feeding deterrency of hydroxamic acids from Gramineae in synthetic diets against the greenbug, *Schizaphis graminum*. *Entomol. exp. appl. 34,* 134-138.

Niemeyer, H. M. (1988): Hydroxamic acids (4-Hydroxy-1,4-benzoxazin-3-ones), defence chemicals in the Gramineae. *Phytochemistry 27*, 3349-3358.

Niemeyer, H. M., E. Pesel, S. Franke and W. Francke (1989): Ingestion of the benzoxazinone DIMBOA from wheat plants by aphids. *Phytochemistry 28*, (in press).

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INTERACTIONS BETWEEN CEREAL APHIDS AND WINTER WHEAT

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Key words: winter wheat, *Rhopalosiphum padi, Sitobion avenae,* glycosidases, pectin esterase, proteases, polyphenol oxidase, peroxidase.

Some enzymes in insects cause changes in substances present in the food plants (Dabrowski, 1988; Nuorteva, 1958). The aim of the present study was to determine the activity and optimum ranges of pH of glycosidases, pectin esterase, proteases, polyphenol oxidase and peroxidase in two species of cereal aphids, *Sitobion avenae* (F.) and *Rhopalosiphum padi* (L.).

The aphids used in the studies were reared in a greenhouse on susceptible winter wheat variety Bezostaya 1. Enzyme activity was estimated in extracts of homogenates of whole aphids - alatae and apterae of both species. The enzymes were determined in appropiate buffer solutions at pH 4.0-9.4, and for proteases the range was expanded to 2.09-9.4. On the basis of the enzymatic reactions rate in broad pH range, the optimum pH range was determined for all enzymes. Enzyme activity was expressed by the number of reaction products in mg, μg, μmol or as ΔA per 1 mg of enzymatic protein. The significance of differences between species and between alatae and apterae were calculated by Student's t-test.

The results obtained indicate that the optimum pH of aphid enzymes varies (Table 1): acid reaction (pH 4.6-6.2) is specific for glycosidases and for pectin esterase, while alkaline reaction (pH 7.0-8.4) for most proteases and for peroxidase. It is different for polyphenol oxidase and ranges from 8.2 to 9.4. Our earlier studies (Niraz et al., 1988; Urbańska and Niraz, 1989) proved that the enzymes in aphids are related to a particular fraction of their saliva.

The two species of aphids differed in the activity of some glycosidases and proteases which reflected the quality and proportion of nutritive components (Fig. 1). The increased activity of α-amylase, trypsin carboxypeptidase A, leucin aminopeptidase was observed in *S. avenae* which feed on assimilative parenchyma (Urbanska and Niraz, 1989). On the other hand, *Rh. padi*, which penetrates through cellwalls into the phloem and absorbs phloem juice, showed greater activity of invertase, pectin hydrolases, cellulase, α- and β-glucosidases. In both species the activity of α-amylase and invertase was greater than the activity of proteases. Differences appeared also in the activities of some glycosidases and proteases between alatae and apterae. A protease with an optimum pH-value between 4.8-5.6 and with specificity

Table 1 Optima of pH ranges and activity of enzymes in homogenates of whole examined cereal aphids

Enzyme	pH range	*S. avenae*				*Rh. padi*			
		apterae		alatae		apterae		alatae	
Polygalacturonase	4.6 to 6.2	4.19	a	8.44	a	7.36	a	9.31	a
			b_1		b_1		a_1		b_1
Pectin esterase		1.11	a	1.52	a	1.59	a	1.80	a
			a_1		a_1		a_1		a_1
α-amylase		0.34	a	0.50	a	0.27	a	0.48	a
			a_1		a_1		a_1		a_1
Celulase		0.04	b	0.06	b	0.07	a_1	0.08	b_1
			a_1		b_1				
α-glucosidase		0.05	a	0.10	a	0.24	a	0.12	a
			a_1		b_1		b_1		b_1
β-glucosidase		0.02	a	0.03	a	0.04	a	0.06	a
			a_1		a_1		a_1		a_1
Invertase		0.29	a_1	0.39	a_1	0.69	a_1	1.02	a_1
Catepsin-D-like enzyme		0.20	a	0.07	a	0.16	a	0.12	a
			a_1		a_1		a_1		a_1
Trypsin	7.0 to 8.4	0.18	a	0.15	a	0.10	a	0.12	a
			a_1		a_1		a_1		a_1
Chymotrypsin		0.34	b	0.32	b	0.33	a	0.35	a
					a_1				a_1
Carboxypeptidase A		0.13	a	0.14	a	0.12	a	0.13	a
			a_1		a_1		a_1		a_1
Carboxypeptidase B		0.21	a	0.24	a	0.20	a	0.25	a
			b_1		a_1		b_1		b_1
Leucin aminopeptidase		15.40	a	4.72	a	5.71	a	1.74	a
			a_1		a_1		a_1		a_1
Peroxidase		0.11	b	0.10	a	0.10	a	0.11	a
			a_1		a_1		a_1		b_1
Polyphenol oxidase	8.0-9.4	0.09	a	0.10	a	0.09	a	0.09	a
					a_1				a_1

a, a_1 - values significantly different at $P \leq 0.01$

b, b_1 - values significantly different at $P \leq 0.05$

a_1, b_1 - differences between aphid species

a, b - differences between the aphid forms

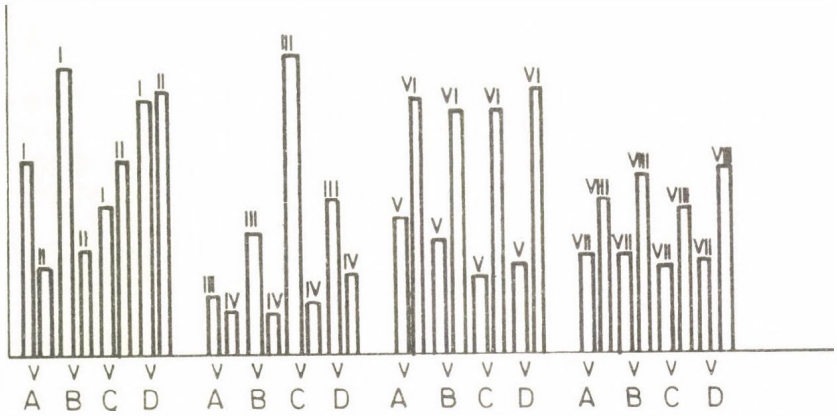

Fig. 1 Diagrammatic comparison of glycosidase and protease activities in cereal aphid homogenates.

I - α-amylase
II - invertase
A - apterate of *S. avenae*
III - α-glucosidase
IV - β-glucosidase
B - alate of *S. avenae*
V - trypsin
VI - chymotrypsin
C - apterate of *Rh. padi*
VII - carboxypeptidase A
VIII - carboxypeptidase B
D - alate of *Rh. padi*

close to pepsin has been discovered in aphids. It is defined as a cathepsin-D-like enzyme. Both aphid species contain enzymes which can neutralize toxic phenols in the wheat tissues.

The results presented above indicate that enzyme activity in cereal aphids, like in other Homoptera (Agarwal, 1975; Dabrowski, 1988; Ishaaya and Swirski, 1976) reflects food quality and nutrient relations. They also indicate that these insects may neutralize harmful metabolites present in host plant tissues.

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REFERENCES

Agarwal, A. K. (1975): Digestive enzymes of sugarcane leafhopper *Pyrilla perpusilla* Wlk. (Fulgoridae: Hemiptera). *Appl. Entomol. Zool. 10,* 140-142.

Dabrowski, Z. T. (1988): *Podstawy Odporności Roślin na Szkodniki.* Wydanie II. PWRIL, Warszawa.

Ishaaya, I. and E. Swirski (1976): Trehalase, invertase and amylase activities in the black scale *Saissetia oleae* and their relation to host adaptability. *J. Insect. Physiol. 22,* 1025-1029.

Niraz, S., A. Urbańska, D. Czyżewska and B. Leszczyński (1988): Some physiological and biochemical relations between cereals and aphids. In: F. Sehnal, D. Denlinger and A. Zabza (eds), *Endocrinological Frontiers in Physiological Insect Ecology.* Proc. Int. Conf., 7-12. September 1987, Szklarska Poręba. Wrocław Technical University Press, *1,* 123-128.

Nuorteva, P. (1958): Die Rolle der Speichelsekrete im Wechselverhältnis zwischen Tier und Nahrungpflanze bei Homopteren und Heteropteren. *Entomol. exp. appl. 1,* 41-49.

Urbańska A. and S. Niraz (1989): Anatomiczne i biochemiczne aspekty żerowania mszyc zbożowych. *Zesz. Probl. Post. Nauk Roln.* (in press).

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SPATIAL VARIATION IN OFFSPRING LIFETIME FITNESS IN AN HERBIVOROUS LADY BEETLE *EPILACHNA NIPONICA*

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Key words: lifetime fitness, oviposition site selection, spatial density-dependence, size-dependent mortality, *Epilachna niponica*.

Epilachna niponica is a specialist herbivore of a thistle plant, and has one generation a year. I have conducted population study of the lady beetle at six different valleys of the River Ado in central Japan. Ohgushi and Sawada (1985) showed that ovipositing females tend to avoid thistles with high egg density, which results in population stabilization in a spatial scale. Thus, the problem which should be addressed next is whether this oviposition site selection improves lifetime reproductive success of ovipositing females. In this context, I propose a hypothesis that lifetime fitness of offspring declines with increasing egg density. The purpose of this study is to test the hypothesis based on direct measure of lifetime fitness of individuals which develop on thistle plants in different egg densities.

Lifetime fitness was divided into six fitness components: egg survival, larval survival, adult survival until hibernation, adult survival during hibernation, reproductive life-span, and lifetime fecundity. To evaluate these fitness components, I used three techniques: life table analysis for immature beetles, mark-recapture experiments for adult beetles, and a laboratory experiment for reproductive females.

Although the egg survival was independent of egg density, the larval survival had a significant negative correlation with cumulative egg density. The size of new adults was found in a decreasing function of leaf damage due to larval feeding which was mostly determined by egg density. Thus, the body size of adults can be seen a suitable indicator of egg density on individual plants on which larvae developed. Both the adult survival until hibernation and the adult survival during hibernation decreased in a size-dependent manner; while the adult size had little effect both on reproductive lifespan and lifetime fecundity.

These findings clearly support the hypothesis proposed above. Namely, individuals developed on thistles with low egg density always enjoy higher lifetime fitness than those on plants with heavy egg load. Consequently, the characteristic oviposition behaviour which avoids plants associated with high egg density has undoubtedly an adaptive significance, thereby improving lifetime reproductive success of ovipositing females.

REFERENCE

Ohgushi, T. and H. Sawada (1985): Population equilibrium with respect to available food resource and its behavioural basis in an herbivorous lady beetle, *Henosepilachna niponica*. *J. Anim. Ecol. 54*, 781-796.

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CELL MEMBRANE PUNCTURES DURING EPIDERMAL PENETRATIONS BY APHIDS: CONSEQUENCES FOR POTATO VIRUS Y TRANSMISSION

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Key words: aphid, electrical penetration graph, membrane puncture, *Myzus persicae*, potato virus Y, stylet activities, virus acquisition, virus inoculation, virus transmission.

Aphids transmit potato virus Y (PVY) and other nonpersistent viruses mainly as a consequence of their brief, epidermal penetrations typically induced by starvation. A knowledge of the route and activities of the stylets during these "probes" is essential for an understanding of the virus transmission processes.

The electrical penetration graph (EPG) signal gives information concerning stylet activities and is produced by making the aphid and plant part of a d.c. circuit (Tjallingii, 1978). This results in the production of a small electric current during recordings and perhaps electrophoresis in the stylet canals (Tjallingii, 1985a). Transmissions of nonpersistent viruses by aphids may depend on electrostatic attachment and release of virus particles to and from their retention site (Vandervecken, 1977). However, when *Myzus persicae* connected to the circuit (mean current = 60 pA) were compared with controls through which no current flowed, acquisition and inoculation of PVY from and to tobacco seedlings during single penetrations were not affected.

A remarkable feature of the EPG is the short potential drop (Tjallingii, 1985b) which has been correlated with the stylet puncture of a cell membrane. This pattern feature was found to be necessary for the acquisition (X^2 = 74.0; P 0.001) and inoculation (X^2 = 7.7; P 0.01) of PVY by *M. persicae*.

REFERENCE

Tjallingii, W. F. (1978): Electronic recording of plant penetration behaviour by aphids. *Entomol. exp. appl. 24,* 721-730.

Tjallingii, W. F. (1985a): Electrical nature of recorded signals during stylet penetration by aphids. *Entomol. exp. appl. 38,* 177-186.

Tjallingii, W. F. (1985b): Membrane potentials as an indication for plant cell penetration by aphid stylets. *Entomol. exp. appl. 38,* 187-195.
Vandervecken, J. J. (1977): Oils and other inhibitors of nonpersistent virus transmission. In: K. F. Harris and K. Maramorosch (eds), *Aphids as Virus Vectors.* Academic Press, New York. 435-454.

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POSITIVE AND NEGATIVE EFFECTS OF HERBIVORY IN THE POPULATION DYNAMICS OF *SENECIO JACOBAEA* AND *CYNOGLOSSUM OFFICINALE*

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Key words: *Cynoglossum officinale*, herbivory, population dynamics, *Senecio jacobaea*, *Tyria jacobaeae*, vegetation cover.

Effects of herbivores on plant populations can be either direct or indirect. Direct effects have received more attention, probably because they are easier to measure.

In the present study, I was primarily concerned with the impact of herbivores on the population dynamics of two biennial species (*S. jacobaea* and *C. officinale*). Plant populations and community structure were monitored in- and outside exclosures during a three year period (1985-1988).

In *S. jacobaea*, a strong negative effect of the monophagous Lepidopteran *Tyria jacobaeae* was found on seedling establishment (Fig. 1, 1986 and 1987), rosette growth and flowering (Fig. 2, cohort 1985).

On the other hand, vertebrate herbivores (mainly rabbits) had an indirect positive effect on these species by limiting the development of the surrounding vegetation (esp. grasses). The increasing vegetation cover in protected populations caused a reduction in germination (Fig. 1, 1988), seedling- and rosette-growth.

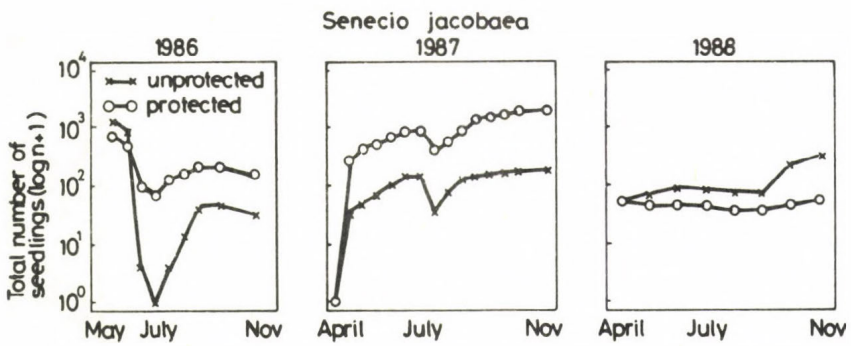

Fig. 1 Number of *S. jacobaea* seedlings from April to November. *=unprotected; o=protected.

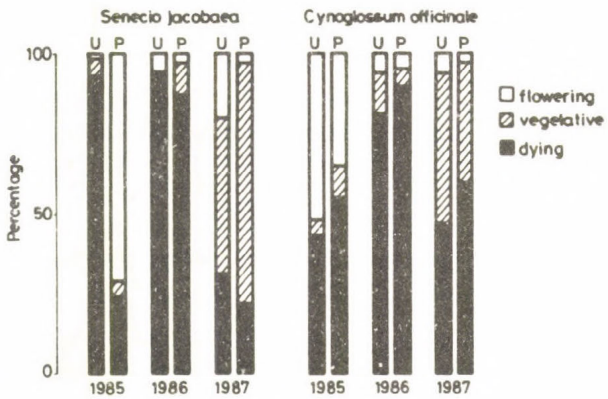

Fig. 2 Fate of the cohorts of plants germinated in 1985 or before, in 1986 or in 1987. U = unprotected, P = protected.

Herbivory on *C. officinale* was low (<10%), no direct effects of insect and vertebrate herbivores on plant populations were found. Indirect effects of rabbits through an increasing vegetation cover were even more pronounced as in *S. jacobaea* (Fig. 3, all three years).

Therefore, although both plant species may first benefit from herbivore-exclusion, their populations are dependent on rabbits eating other plants (esp. grasses) and reducing competition.

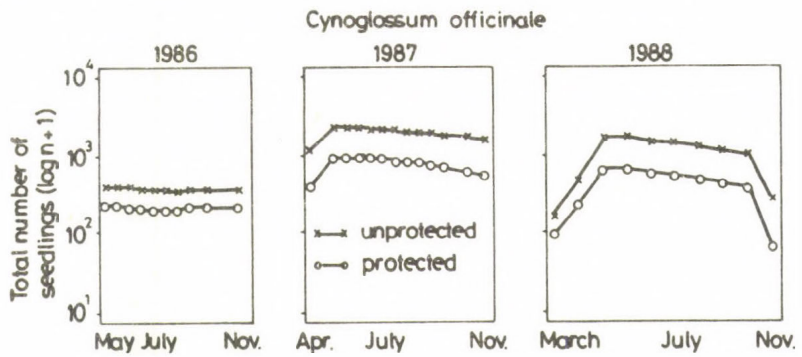

Fig. 3 Number of *C. officinale* seedlings from April to November. *=unprotected; o=protected.

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NOVEL APPROACHES TO THE DEVELOPMENT OF *SORGHUM* GERMPLASM RESISTANT TO GREENBUGS

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Key words: *Schizaphis graminum*, antibiosis, antixenosis, tolerance, *Sorghum halepense*, insect biotypes, toxin.

The greenbug, *Schizaphis graminum* (Rondani) has been a serious pest of sorghum since 1968 when biotype C arose (Harvey and Hackerott, 1969). Now at least eight biotypes (A-H) are documented, usually by their ability to damage plant genotypes (Puterka et al., 1988). We are studying the underlying mechanisms of host plant resistance in order to produce a more durable form of resistance. As we gain more knowledge, perhaps "...we will be able to approach the goal of developing agronomic plants that are deliberately and foresightedly designed to be insect-resistant" (Beck, 1965).

Detailed analysis of traditional mechanisms. By understanding the host plant resistance mechanisms of antibiosis, antixenosis, and tolerance, we have come at least a step closer the "...deliberately and foresightedly designed..." plant. Bramel-Cox et al. (1986) found reasonable levels of antibiosis in sorghum lines IS2238 (actually IS2388) and PI266965, antixenosis in IS5300 and J242, and tolerance in PI229828. Thus, using combinations of these lines could possibly yield a line with an exceptionally effective set of characteristics. Unfortunately, PI266965 is a tetraploid *Sorghum halepense* plant, and thus, resistance would have to be transferred across a ploidy barrier. This is currently being attempted by P. Bramel-Cox and G. H. Liang at Kansas State University (Bramel-Cox et al., 1986).

Two other points need to be made regarding the findings of Bramel-Cox et al. (1986). First, when correlations were calculated for mechanisms of 12 resistant lines versus their score in a traditional flat screening test, the average damage score correlated most closely with antibiosis rather than tolerance, as might have been predicted intuitively. Thus, we have apparently been screening for antibiosis rather than tolerance. The only tolerant line, PI229828, was actually 100 times more tolerant then the second most tolerant line, indicating the small number of sources for tolerance, as identified by the standard screening techniques.

The details of measuring tolerance are critical. Tolerance, by definition, should put less selection pressure on the insect population for new biotypes than antibiosis or even antixenosis. It also should be less likely to have any deleterious effects on

natural enemy (third trophic level) populations. And yet, it is not easy to compare the tolerance of a plant exhibiting antibiosis with one that shows little antibiosis, since by the end of the experiment, the former will have had to "tolerate" many fewer insects than the latter. These difficulties in making valid comparisons have been partially overcome by devising a tolerance index (Dixon, 1988; Dixon et al., 1989b), into which both antibiosis and plant genotype to genotype differences are factored. It still is not ideal, because the number of insects may be changing throughout the experiment. The tolerance index as calculated by Dixon et al. (1989b) is:

$$(((CP - IP)/CP)*100)/TGB$$

where CP = the above-ground dry weight of the control plant, IP = the above-ground dry weight of the infested plant, and TGB = the total number of greenbugs on the infested plant at the end of the experiment.

Use of toxin. As pointed out above, improved techniques for examining tolerance will enhance our efforts to utilize tolerance in our host plant resistance program, thus enabling us to release material that will place less selection pressure on greenbug populations for new biotypes. Although the techniques described above quantify tolerance while taking into account factors, such as plant genotype to genotype differences, as well as antibiosis levels, assessment would be still more direct at a more biochemical level, separate from the feeding insect altogether. We have clearly demonstrated the existence of a toxin, separate and apart from feeding insects, and have used toxin mimics to assess tolerance (Schmidt, 1987). We also have shown a clear correlation between tolerance, as measured by Dixon et al. (1989b), and the formation of a red spot. A susceptible hybrid had a control to treatment ratio of 34.8, whereas PI229828 had a control to treatment ratio of 2.4, when treated with a toxin mimic and measured with a reflectance spectrophotometer (Schmidt, 1987). Current work is concentrating on the isolation of the toxin from greenbugs and detection of toxic materials in greenbug extracts, salivary glands, and at the feeding site. Emphasis is being placed on pectin methylesterase and polygalacturonase. Enzyme-specific stains and appropriate electrophoresis gels are being employed.

IFM and computerization. Ever since McLean and Kinsey (1964) developed a system for electronically monitoring aphid feeding behavior, the possibilities for quantifying behavior after host location, in places not visible for observation, have been nearly endless. More recently, the technique has been utilized to better understand the details of host plant resistance mechanisms (Campbell et al., 1982).

We have utilized modifications of the IFM to distinguish behavioral differences of insects on greenbug-susceptible vs. greenbug-resistant sorghum varieties (Dixon et al., 1989a). An advantage of this system in a host plant resistance program is that it is nondestructive to the plant; if an individual plant looks especially promising, it can be grown to maturity. Further, individual to individual differences can be assessed in both the plant population and the insect population. Finally, although the pectin-pectinase relationships in aphid-host interactions (Dreyer and Campbell, 1984, 1986) have received a good deal of attention and appear to explain resistance levels in some varieties, other resistance mechanisms may occur as well. After finally reach-

ing the phloem, a greenbug may be more likely to pull out quickly from a resistant germplasm accession than from a susceptible accession. This behavior may be due to compounds in the phloem and/or some type of wound response by the plant that plugs the phloem. Such relationships can be detected with the IFM.

We have recently computerized the acquisition of data, resulting in a financial savings on the purchase of strip chart recorders, and have written software to assist in analysis of the data (Noyes et al., 1989). The system utilizes a Zenith Z-158 microcomputer with a 20 MB hard disk and a MetraByte Dash-8 12 bit analog to digital converter board.

Utilizing a variety of approaches has enabled us to make progress toward breeding more durable sources of greenbug-resistant sorghum and has perhaps brought us a little closer to sorghum varieties that are "...deliberately and foresightedly designed..." to be resistant to greenbugs.

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REFERENCES

Beck, S. D. (1965): Resistance of plants to insects. *Ann. Rev. Entomol. 10*, 207-232.

Bramel-Cox, P. J., A. G. O. Dixon, J. C. Reese and T. L. Harvey (1986): New approaches to the identification and development of sorghum germplasm resistant to the biotype-E greenbug. *Proc. 41st Ann. Corn Sorgh. Res. Conf. Amer. Seed Trade Assoc.,* Washington, D. C. *41*, 1-16.

Campbell, B. C., D. L. McLean, M. G. Kinsey, K. C. Jones and D. L. Dreyer (1982): Probing behavior of the greenbug (*Schizaphis graminum*, biotype C) on resistant and susceptible varieties of sorghum. *Entomol. exp. appl. 31,* 140-146.

Dixon, A. G. O. (1988): *Mechanisms and Inheritance of Resistance to Greenbug (*Schizaphis graminum *(Rondani)), Biotype E in Sorghum (*Sorghum bicolor *(L.) Moench).* Ph.D. Dissertation, Kansas State University, Manhattan, Kansas.

Dixon, A. G. O., P. J. Bramel-Cox, and J. C. Reese (1989a): Feeding behavior of biotype E greenbugs (Homoptera: Aphididae) and its relationship to resistance in sorghum. *J. econ. Entomol.* (In Press).

Dixon, A. G. O., P. J. Bramel-Cox and J. C. Reese (1989b): Mechanisms of resistance and their interactions in twelve sources of resistance to biotype E greenbugs (Homoptera: Aphididae) in sorghum. *J. econ. Entomol.* (In Press).

Dreyer, D. L. and B. C. Campbell (1984): Association of the degree of methylation of intercellular pectin with plant resistance to aphids with induction of aphid biotypes. *Experientia 40*, 224-226.

Dreyer, D. L. and B. C. Campbell (1986): Chemical basis of host-plant resistance to sap-feeding insects. *Rev. Latinoamer. Quim. 17*, 204-207.

Harvey, T. L. and H. L. Hackerott (1969): Recognition of a greenbug biotype injurious to sorghum. *J. Econ. Entomol. 62*, 776-779.

McLean, D. L. and M. G. Kinsey (1964): A technique for electronically recording aphid feeding and salivation. *Nature 202*, 1358-1359.

Noyes, S., D. C. Margolies, J. C. Reese, A. G. O. Dixon and P. Bramel-Cox (1989): Computer acquisition and analysis of insect feeding monitor data. (In preparation).

Puterka, G. J., D. C. Peters, D. L. Kerns, J. E. Slosser, L. Bush, D. W. Worrall and R. W. McNew (1988): Designation of two new greenbug (Homoptera: Aphididae) biotypes G and H. *J. Econ. Entomol. 81*, 1754-1759.

Schmidt, D. J. (1987): *The Physiology of Greenbug Feeding Behavior and the Effect of Salivary Toxins on Sorghum Plants.* Ph.D. Dissertation, Kansas State University, Manhattan, Kansas.

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TOLERANCE OF CARDENOLIDES IN *ERYSIMUM CHEIRANTHOIDES* BY THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA*

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Key words: *Erysimum cheiranthoides, Plutella xylostella, Pieris rapae,* cardenolides, diamondback moth, cabbage butterfly, oviposition, oviposition deterrents, oviposition stimulants, Cruciferae.

Many plants are protected from insects by the presence of toxic constituents. These, or associated compounds, may serve to warn potential invaders by functioning as oviposition or feeding deterrents. Oviposition deterrents to the cabbage butterfly, *Pieris rapae*, were recently detected in an unacceptable wild crucifer, *Erysimum cheiranthoides* (Renwick and Radke, 1985). This plant appears to be effectively protected from *P. rapae*, despite the presence of oviposition stimulants (Renwick and Radke, 1987). The deterrents have been characterized as cardenolides (Renwick et al., 1989), but specific structural features are necessary for activity. The most active cardenolides were identified as erysimoside and erychroside (Sachdev-Gupta et al., 1989).

The diamondback moth, *Plutella xylostella*, has a host range similar to that of *P. rapae*, i.e., primarily crucifers. Experiments were therefore performed to determine whether *P. xylostella* would also be deterred by extracts of *E. cheiranthoides*. Bioassays were conducted with approx. 30 adults (mixed sexes) in 48 x 48 x 48 cm screened cages under fluorescent lights that provided a 16:8 (L:D) photoperiod. For each replication, one treated and one control cabbage plant were placed in opposite corners of each cage before the onset of the dark period, and eggs were counted the next morning. Treated plants were sprayed with 5 gram leaf equivalents of *E. cheiranthoides* butanol extract in MeOH:H_2O (50:50). Control plants were sprayed with the same volume (5 ml) of 50 % MeOH alone.

The results (Table 1) showed that the cardenolide-containing butanol extract of *E. cheiranthoides* had no deterrent activity against *P. xylostella*. In fact, a distinct stimulatory effect was indicated, as twice as many eggs were laid on treated plants.

Further experiments were designed to examine the utilization *E. cheiranthoides* by *P. xylostella*. When offered the plants, adults readily laid eggs, and the larvae fed and developed normally. Frass was collected from larvae feeding on *E. cheiranthoides* by attaching paper collars under the foliage. Frass and pupae obtained from these plants as well as fresh foliage were analyzed for cardenolides by the previously described isolation scheme (Renwick et al., 1989), using HPLC of the butanol extracts for the final comparisons. The chromatograms clearly showed that the major

Table 1 Oviposition by *Plutella xylostella* on cabbage treated with butanol extract of *Erysimum cheiranthoides* (T) or with solvent alone (C).

Test No.	No. of eggs laid	
	T	C
1	388	315
2	287	73
3	252	110
4	353	132
5	258	134
6	139	58
7	93	17
8	23	5
9	188	114
10	40	0
Total	2021	958

cardenolides, erysimoside and erychroside, are present in the frass in almost the same proportions as in the original foliage. No cardenolides were detected in the pupae from larvae that completed their development on these plants.

The results show that diamondback moths differ dramatically from cabbage butterflies in their response to cardenolide-containing extracts of *E. cheiranthoides*. Oviposition is not deterred, but is actually stimulated by unknown constituents in the extracts. *P. xylostella* apparently tolerates the relatively high levels of cardenolides by rapid excretion, without detectable assimilation or alteration of these potentially toxic compounds.

REFERENCES

Renwick, J. A. A. and C. D. Radke (1985): Constituents of host and non-host plants deterring oviposition by the cabbage butterfly, *Pieris rapae. Entomol exp. appl. 39,* 21-26.

Renwick, J. A. A. and C. D. Radke (1987): Chemical stimulants and deterrents regulating acceptance or rejection of crucifers by cabbage butterflies. *J. Chem. Ecol. 13,* 1771-1775.

Renwick, J. A. A., C. D. Radke and K. Sachdev-Gupta (1989): Chemical constituents of *Erysimum cheiranthoides* deterring oviposition by the cabbage butterfly, *Pieris rapae. J. Chem. Ecol. 15,* 2161-2169.

Sachdev-Gupta, K., J. A. A. Renwick and C. D. Radke (1989): Isolation and identification of cardenolides from *Erysimum cheiranthoides* that deter oviposition by *Pieris rapae. J. Chem. Ecol.* (in press).

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INFLUENCE OF SHAPE, SIZE AND COLOUR OF SURROGATE LEAVES ON OVIPOSITION BEHAVIOUR OF *DELIA RADICUM*

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Key words: *Delia radicum*, cabbage fly, oviposition, surrogate leaf, colour shape, size, stem, veins

Host finding and acceptance in insects is generally viewed as a chain of steps. The progression through the chain is a function of sensory cues perceived by the insect. While the importance of chemical stimuli has been emphasized, it has become clear that interactions with other modalities also play a role (Miller and Strickler, 1984). Insight in these processes is needed as a basis for attempts to manipulate insect-plant relations. For the cabbage fly *Delia radicum* only cues used in the first part of the behavioral chain (i.e. for host finding) have been investigated in detail (Prokopy et al., 1983; see Nothingham, 1988 for review). Effects of non-chemical cues on oviposition have not been systematically tested in this species.

A surrogate leaf for oviposition assays was described by Städler and Schöni (in press). When sprayed with an ethanolic extract of cabbage it proved to be as acceptable as a real cabbage leaf. We investigated the influence of non-chemical factors on this acceptability by systematically evaluating the effects of shape, size, colour, surface cover and the presence of a stem on oviposition.

To allow convenient manipulation of cues we used paper models in the oviposition assay. Each model was sprayed with an extract of cabbage leaves to provide sufficient stimulation for oviposition. The models under investigation were placed in a circle on the bottom of the cage for a 24 h period. After this period the eggs layed were counted.

From the set of tested colours (blue, red, yellow and shades of green) bright green leaves yielded better results then red or blue ones, but yellow leaves were not significantly different. Changing the shade of green had only a minor influence. Shapes of equal size were not discriminated but egg counts increased with surface area and with the presence of a stem. Only folded three dimensional models were as effective as the original plastic leaf. Behavioural observations showed that on leaves with horizontal folds flies spend more time in exploration and significantly fewer flies complete the behavioural chain. The stimulating effect of vertical folds is due to vertical edges that guide the flies during the characteristic geotactic leaf and stem run.

REFERENCES

Städler, E. and R. Schöni. Oviposition behaviour of the cabbage root fly, *Delia radicum* (L), influenced by host plant extracts. *J. Insect Behav.* (in press)

Miller, J. R. and K. L. Strickler (1984): Finding and accepting host plants. In: W. J. Bell and R. T. Cardé (eds), *Chemical Ecology of Insects.* Chapman and Hall, London. 127-158.

Nottingham, S. F. (1988): Host-plant finding for oviposition by adult cabbage root fly, *Delia radicum. J. Insect Physiol. 34,* 227-234.

Prokopy, R. J., H. Collier and S. Finch (1983): Visual detection of host plant by cabbage root flies. *Entomol. exp. appl. 34,* 85-89.

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IMPACT OF THE ROOT MINER, *ETEOBALEA SERRATELLA* TR. (LEP., COSMOPTERIGIDAE), ON REPRODUCTION OF THE WEED, *LINARIA VULGARIS* (SCROPHULARIACEAE)

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Key words: *Eteobalea serratella, Linaria vulgaris,* biological control, below-ground herbivory, biomass.

L. vulgaris is a perennial which became a serious weed in western North America after its introduction from Europe (McClay, 1989). The moth *E. serratella*, a univoltine miner of the central root system, has been under investigation for use in classical biological control at our station since 1987. The experiment was carried out to:

1) estimate the impact of root mining on individual plant performance,

2) provide a basis for monitoring and understanding the effects on plant population dynamics after its introduction into North America, and

3) contribute information concerning the effects of below-ground herbivory, which is poorly understood (Andersen, 1987).

Field collected plants were potted as single tillers (N=40), after the fresh weight was measured. Half of them received three first instar larvae of *E. serratella* July 20, 1988. Fruits were cut as soon as they were completely ripe. Above ground biomass was measured Nov. 3, 1988. Infestation success and total biomass were determined June 6, 1989. Plants were kept in a greenhouse during the flowering season, where they were kept relatively dry, and overwintered in the garden. Two of each of the attacked and unattacked plants did not survive the summer, and were excluded. The remaining plants all survived the winter. Fourteen plants were successfully attacked by one larva in spring 1989 (with a single exception). This is representative of natural density.

Fruit and seed production were correlated with initial weight. Weights were distributed equally between test and control groups. Total numbers of fruits and seeds were lower in attacked plants, but did not differ significantly. However, the average weight of a single seed was significantly lower in attacked plants. [One way ANCOVA; main effect (attack): $F = 7.51$, d.f. $= 1.22$, $p = 0.012$, covariate (initial fresh-weight): $F = 7.75$, d.f. $= 1.22$, $p = 0.011$)].

The flowering and fruit production periods were shorter for attacked plants. Number and weight of stems produced during the flowering season were not influenced significantly. Total weight of stems and roots, as well as total biomass produced, were lower for attacked plants, but did not differ significantly. More stems (1), however, with a lower average weight (2) were produced in spring. [One way ANCOVA, main

effect (attack): F = 9.23, d.f. = 1.28, p = 0.005 (1); F = 5.62, d.f. = 1.28, p = 0.025 (2); covariate (initial freshweight): F = 0.21, d.f. = 1.28, p = 0.65 (1); F = 3.75, d.f. = 1.28, p = 0.063 (2)].

Apparently the apical dominance of the first shoot is broken by root crown feeding. The effects on the host plant population are not possible to estimate as the compensatory capacity of this long lived plant species is unknown. The results suggest, however, that continuous attack negatively influences plant performance as well as the likelihood of successful seedling establishment.

REFERENCES

Andersen, D. C. (1987): Below-ground herbivory in natural communities: a review emphasizing fossorial animals. *Quart. Rev. Biol. 62,* 261-286.

McClay, A. S. (1989): *Selection of Suitable Target Weeds for Classical Biological Control in Alberta.* Alberta Environmental Centre, Vegreville.

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CONCORDANCE OF TREE QUALITY AMONG SPECIES OF THE LEAF-CHEWING INSECT GUILD ON MOUNTAIN BIRCH

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Key words: *Betula pubescens* ssp. *tortuosa*, competition, *Epirrita autumnata*, guild, relative growth rate, Symphyta

The mountain birch (*Betula pubescens* ssp. *tortuosa* (Ledeb.) Nyman) shows extensive morphological variation in the northernmost part of its distribution (Vaarama and Valanne, 1973), and contains a heavy load of insect herbivores (Koponen, 1983). Two investigations were made: upon the variability in space and time of a plant population in terms of food quality for herbivores; and upon the effects of variability in quality of host plants upon different members of the herbivorous insect guild.

Twenty marked mountain birches in subarctic Finland were tested over two seasons in short-term growth trials with 15 naturally occurring leaf-chewing insect species: *Epirrita autumnata* (Bkh.) (Lep.: Geometridae) (see Haukioja et al., 1988); and 14 species of sawflies (Hym.: Symphyta). In laboratory, the growth trials were conducted on detached leaves during the same periods as the herbivores occurred naturally, upon their host plants. Mean relative growth rates, over 24 hours of individually-kept larvae, were determined for each tree. When the tree-specific growth rates of different species were compared, it was found that, in the first year, 13.3% and in the second year 41.8% of all possible species-pairings were significantly ($p < 0.05$) correlated. All significant, or close to significant, correlations were positive. In the first season, a year with generally high tree-quality, significant correlations were observed between species occurring in the first half on the season, whereas in the second year, with lower tree quality, significant correlations were found during the entire season. When comparing growth rates of each herbivore species between the two years, only 2 significant correlations were found, in *E. autumnata*, as the first herbivore in the season, and in an early sawfly species. The growth rates of all species pooled on individual trees were significantly correlated between the two years ($p = 0.004$). This indicates that the quality of individual trees remains constant for the guild.

The lack of any negative correlations shows that all species were similarly affected by the quality of their host trees. There appears to be no selection pressure (e.g., competition) acting upon the guild members to specialize toward different "diet types" of their host species.

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REFERENCES

Haukioja, E., S. Neuvonen, S. Hanhimäki and P. Niemelä (1988): The autumnal moth in Fennoscandia. In: A. A. Berryman (ed), *Dynamics of Forest Insect Populations: Patterns, Causes and Management Strategies.* Plenum Press, New York, 166-178.

Koponen, S. (1983): Phytophagous insects of birch foliage in northernmost woodlands of Europe and eastern north America. *Nordicana 47,* 165-176.

Vaarama, A. and T. Valanne (1973): On the taxonomy, biology and the origin of *Betula tortuosa* Ledeb. *Rep. Kevo Subarct. Res. Stat. 10,* 70-84.

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NUTRITIONAL ECOLOGY OF THE MONOPHAGOUS CINNABAR MOTH

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Key words: *Tyria jacobaeae, Senecio jacobaea,* fitness, pupal weight, food quality, jacobine-alkaloids, senecionine-alkaloids.

In the coastal sand dune area of Meyendel (near The Hague, the Netherlands) the numbers of the monophagous cinnabar moth (*Tyria jacobaeae*) are frequently limited by the quantity of food (ragwort, *Senecio jacobaea*). This does not mean that food quality is unimportant, for ovipositing females select plants with both high protein and sugar concentrations (van der Meijden et al., 1989). Therefore, we studied the relationship between these nutrients and herbivore fitness.

Pupal weight was used as a fitness parameter, because it is strongly positively related to winter survival and fecundity. In two years fifth (final) instar caterpillars were collected in the field and reared in the laboratory untill pupation. Leaves of the plants from which they had eaten in the field were sampled for chemical analyses.

In both years pupal weight was not correlated with sugar concentration. The correlation with protein concentration was positive in 1987 and negative in 1988 (Table 1). Mean pupal weight was much higher in 1988.

The completely opposite relationship between pupal weight and protein concentration in the two years suggests that another factor is more important. A likely explanation was found in the secondary metabolites of the pyrrolizidine alkaloid group. The concentration of the jacobine-group was positively correlated with pupal weight in both years (Table 1). These alkaloids are very specific for ragwort. Three other alkaloids, which are less specific for ragwort (the senecionine-group) were in a significant positive correlation only with pupal weight in 1987. Secondary meta-

Table 1 Correlation coefficients between food quality and pupal weight.

	Correlation coefficients	
	1987	1988
Sugar	-0.130 (14)	-0.035 (14)
Protein	0.732** (25)	-0.516* (25)
Jacobine-alk.	0.996** (4)	0.983* (4)
Senecionine-alk.	0.985* (4)	0.573 (4)

*=p<0.01; **=p<0.005

bolites may have a phagostimulating effect on this specialist species and thereby positively influence growth. Similar results in another species were found by Berenbaum (1981).

REFERENCES

Berenbaum, M. (1981): Effects of linear furanocoumarins on an adapted specialist insect (*Papilio polyxenes*). *Ecol. Entomol. 6,* 345-351.

Meijden, E. van der, A. M. van Zoelen and L. L. Soldaat (1989): Oviposition by the cinnabar moth, *Tyria jacobaeae,* in relation to nitrogen, sugars and alkaloids of ragwort, *Senecio jacobaea. Oikos 54,* 337-344.

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THE IMPACT OF THE SELF-INTRODUCED TWIGMINING MOTH *LEUCOPTERA SPARTIFOLIELLA* ON BROOM (*CYTISUS SCOPARIUS*) IN NEW ZEALAND

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Key words: *Leucoptera spartifoliella*, biological control, *Cytisus scoparius*, twigmining moth.

The broom twigminer *Leucoptera spartifoliella* (Hübner) (Lepidoptera: Lyonetiidae), a species of European origin, is now widely distributed in New Zealand, and is found on broom, *Cytisus scoparius* L. (Link) in most parts of the country (Scheele and Syrett, 1987). The moth was first recorded here in 1950 as an accidental introduction which probably gained entry on imported ornamental broom plants.

L. spartifoliella was introduced into the United States in the early 1960s for biological control of broom (Frick, 1964). This followed laboratory testing of its potential host range in France which showed it to be highly specific to *Cytisus* species. Had the moth not been already present in New Zealand, it would have been a primary candidate for introduction under our current programme for the biological control of broom.

The life cycle of *L. spartifoliella* in New Zealand is similar to that recorded in Europe. Adults occur in field from December to March. Eggs are laid on green stems, the new growth being preferred. Hatching larvae mine the chlorenchymatous tissue, and overwinter as larvae. In spring, wandering sixth instar larvae spin cocoons, usually attached to the underside of branches, in which they pupate. The pupation stage lasts about one month.

Populations of *L. spartifoliella* frequently reach high levels, and larval mining results in the production of considerable areas of dead wood, which may accelerate the demise of older broom bushes. One possible factor contributing to the large numbers of moths is the apparent absence of parasitism, particularly of the eulophid *Tetrastichus evonymellae* (Bouché) to which Agwu (1967) attributed a probable controlling influence on populations in England. Our experiments to measure fecundity of *L. spartifoliella* under fluctuating temperatures (mean 21 °C) gave a mean of 95 ± 20 eggs per female, which is comparable to values obtained by Agwu (1967) at 20 °C.

In order to assess the proportion of broom twigs killed by larval mining, two field sites were selected where *L. spartifoliella* was known to occur in good numbers. At each of these sites, in September 1988 (southern hemisphere spring), 300 randomly selected live twigs, approximately 150 mm long, were cut from ten broom bushes. They were then dissected for *L. spartifoliella* larvae. At the same time as the twigs were cut, 300 more were tagged with plastic covered twist-ties. In late January 1989

Table 1 Percentage of 150 mm long broom twigs containing *Leucoptera spartifoliella* larvae or showing typical evidence of mining from September sample.

Site	Calf Stream (near Hanmer, North Canterbury)	Hoon Hay Valley (Port Hills, Christchurch)
% twigs mined	83	89
% twigs with 3 or more larvae	14	12
% twigs with 2 or more larvae	35	29
% twigs with 1 or more larvae	61	57
% twigs died (January sample)	31	30

Table 2 The percentage of living and dead twigs with larval mines at two sites in New Zealand in January compared with figures from southern England.

	New Zealand (mean from 2 sites)	England (Agwu 1967)
% living twigs with mines	95	61.1
% dead twigs with mines	100	95.5

(summer) the tagged twigs were collected and assessed as to whether they were alive or dead. Twigs were also assessed for the presence of larval mining.

Ninetythree percent of tagged twigs were retrieved from the Calf Stream site but only 61% from Hoon Hay Valley. This was the result of browsing by sheep which removed some of the twigs during a period of very dry weather.

From both the September (Table 1) and January (Table 2) samples, a higher proportion of twigs overall was found to be mined at these two sites than in Agwu's study in England. The proportion of dead twigs in the sample tagged in September, and collected in January, was assumed to be the same as the proportion of twigs cut in September, which would have been expected to die, had they remained on the bushes until January. Thus of twigs that were mined, 33% died; 30% of twigs died overall. Up to six larvae were dissected from a single twig, and the mean number of larvae per 150 mm long twig was 1.1 ± 1.0 for the two sites combined. The percentage of twigs, it is assumed, would have died by January from the sample cut in September, corresponded most closely to the percentage of twigs from which two or more larvae were dissected (Table 2).

While *L. spartifoliella* demonstrably can cause death of much of the current year's growth through twigmining (Fig. 1), we have not yet shown how this affects the lifespan and mortality of whole bushes or the effect on the next season's flowering. If *L. spartifoliella* is exerting a significant effect on its host plant, it may contribute towards successful biological control of broom in conjunction with new introductions into New Zealand of further host specific insects. The apparent absence of parasitism may be responsible for higher levels of damage than occur in Europe.



Fig. 1 The impact of larval mining by *Leucoptera spartifoliella* on broom twigs (a) typical twig tagged in September, (b) dead twig (mined) cut in January, (c) twig showing no growth in January, (d) twig showing growth.

REFERENCES

Agwu, S. I. (1967): *The biology and population dynamics of* Leucoptera spartifoliella *Hübner on broom* Sarothamnus scoparius. PhD Thesis, University of London.

Frick, K. E. (1964): *Leucoptera spartifoliella,* an introduced enemy of Scotch broom in Western United States. *J. econ. Entomol. 57,* 566-569.

Scheele, S. M. and P. Syrett (1987): The broom twigminer, *Leucoptera spartifoliella* (Lepidoptera: Lyonetiidae), in New Zealand. *N. Z. Entomol. 10,* 133-137.

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ENVIRONMENTAL EFFECTS ON PRODUCTION OF A PLANT SYNOMONE THAT ATTRACTS PREDATORY MITES

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Key words: Acarina, Phytoseiidae, *Phytoseiulus persimilis*, tritrophic interactions, infochemical, synomone, light intensity, environmental effect, olfactometer, indirect defence

Spider mites are very ravenous herbivores that overexploit their host plants. However, when discovered by predatory mites, populations of the herbivore can be decimated. Thus, plants that harbour predatory mites are at a clear advantage. It is known that plants are involved in attraction of predatory mites *after* infestation by spider mites occurs (Dicke et al., 1989). In this paper, we report that plants may also attract predatory mites *before* spider mites inflict damage.

Predator: satiated female *Phytoseiulus persimilis* reared on two-spotted spider mite (*Tetranychus urticae*) on Lima bean. *Plants:* Lima bean, cucumber, tomato, *Solanum luteum,* and *S. dulcamara. Olfactometer:* A Y-tube olfactometer was used.

1. Do plants attract predatory mites before spider mites inflict damage? In the olfactometer we offered leaves of uninfested plants vs. clean air. Moist cotton wool was added in each arm. *P. persimilis* is attracted by an allelochemical emitted by uninfested Lima bean leaves and tomato leaves, but not by the other plant species tested (Table 1).

2. Are there environmental effects on production of the volatile synomone? (2-a) Effects of light intensity on synomone production by Lima bean plants? Plants were reared in green house (15 days) and subsequently placed at different light intensities. Leaves of plants placed at high light intensity (7000 lux) are more attractive than leaves of plants placed at low light intensity (2000 lux) (Chi-square test, $P < 0.05$, $n = 153$).

(2-b) Effect of time of the year on synomone production? Experiment (2-a) was carried out at different times of the year. The effect of light intensity, experienced by plants during 6 days prior to the experiment on predator response depends on the time of the year at which plants were reared (Fig. 1).

Table 1 Percent response of *P. persimilis* to intact plants vs. clean air.

Lima bean	Cucumber	Tomato	*S. liteum*	*S. dulcamara*
67.5* (80[1])	46.3[ns] (80)	60.6* (80)	56.3[ns] (80)	44.8[ns] (66)

[1] Number of replications. Significance of difference from 50%, Chi-square test; *:$p<0.05$, ns: not significant

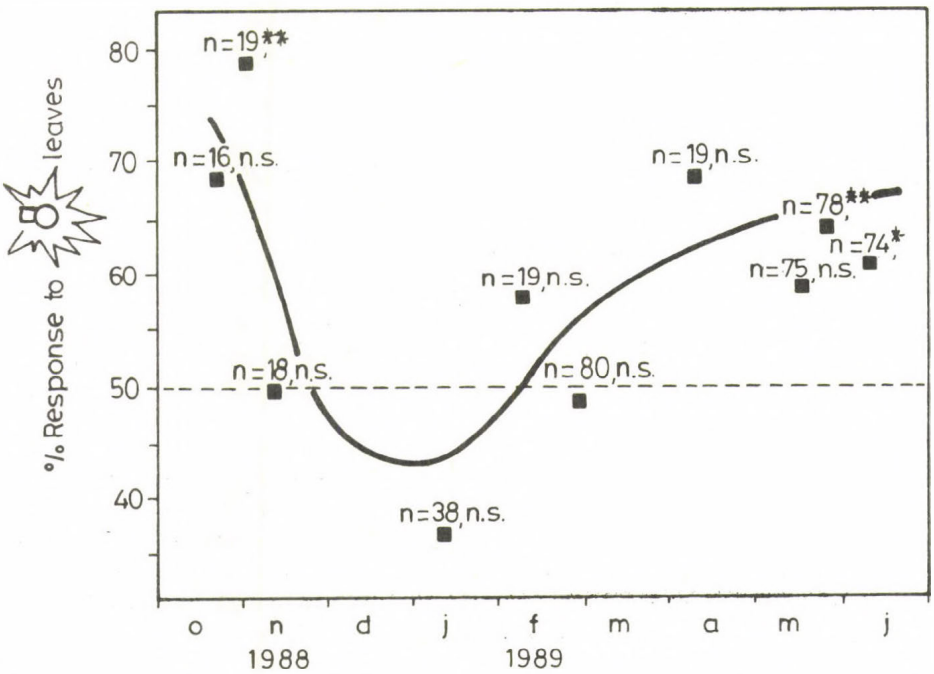

Fig. 1 Percent response to the highly lit leaves. n.s.=P>0.05; *=P<0.05; **=P<0.01.

Thus, plants may recruit their herbivores' natural enemies before attack by the herbivores, but whether they do so depends on the plant species and the condition of the plants.

REFERENCE

M. Dicke, T. A. van Beek, M. A. Posthumus, N. Ben Dom, H. van Bokhoven and Æ. de Groot (1989): Isolation and identification of a volatile kairomone that affects acarine predator-prey interactions: involvement of host plant in its production. *J. Chem. Ecol. 15,* (in press)

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NEGATIVE INFLUENCE OF INFESTED HOST PLANTS ON SPIDER MITE FEEDING AND POPULATION DEVELOPMENT

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Key words: *Tetranychus urticae*, population dynamics, plant defense, fecundity, feeding, cucumber, *Chrysanthemum*, strawberry, ^{14}C labelling.

It was found earlier that spider mites can induce some negative reactions in their host plants which subsequently influence mite behaviour (Karban and Carey, 1984; Harrison and Karban, 1986). To study this phenomenon some experiments were conducted on two varieties of cucumber, Atos and Wilanowski cultivated in the greenhouse. Food uptake from leaves labelled with ^{14}C was studied. In one group of plants non-infested leaves were labelled, on which spider mites were feeding 5 days previously. The mites were removed from the damaged leaf prior ^{14}C labelling. New females were transferred to radioactive leaves of both groups of plants and left there for 24 h. After this period radioactivity of 100 females feeding on non-infested and on infested leaves was compared. Intensity of spider mite feeding was strongly decreased on infested leaves as compared to non-infested ones. This phenomenon can be connected with a defence response of the host plants i.e., changes in the chemical composition of infested leaves which render them less suitable for the feeding of spider mites. After a short period of spider mite feeding on *Chrysanthemum* and strawberry, a decrease of sugar concentration and changes in phenol content in infested leaves was found (Kiełkiewicz and van de Vrie, 1983; Tomczyk et al., 1987; van de Vrie et al., 1988).

Fecundity of females of *Tetranychus urticae* on infested and on non-infested leaves of cucumber were also studied. Three young females were transferred on leaf disks in petri dishes or to the cages on the underside of the leaf. The number of eggs was counted after 3 days. Egg production by females feeding on previously infested leaves was lower as compared to non-infested ones. This can be related to the decrease in food uptake from injured leaves.

Development of *T. urticae* population was studied on the plants of variety Atos. The initial mite population was 10 or 20 mites per plant. The number of mites was counted after 2.4 and 7 weeks. During the first 4 weeks of feeding population development was restricted. This probably resulted from a negative influence of the injured plant on food uptake and egg production of the pest, induced by mites in the

initial period of feeding. A negative influence of infested plant on spider mites was also observed on cotton (Karban and Carey, 1984; Harrison and Karban, 1986).

REFERENCES

Harrison, S. and R. Karban (1986): Behavioural response of spider mites (*Tetranychus urticae*) to induced resistance of cotton plant. *Ecol. Entomol. 11,* 181-188.

Karban, R. and J.R. Carey (1984): Induced resistance of cotton seedlings to mites. *Science, 225,* 53-54.

Kiełkiewicz M. and M. van de Vrie (1983): Histological studies on strawberry leaves damaged by the two-spotted spider mite (*Tetranychus urticae* Koch). Some aspects of plant self defence. *Med. Fac. Landbouww. Rijksuniv. Gent, 48,* 235-245.

Tomczyk A., D. Kropczyńska and M. Kiełkiewicz (1987): Symptoms of resistance to spider mites in various cultivated plants. *Mat. XXVII Sesji Nauk. IOR, Part I.* 65-79. (In Polish with English summary).

van de Vrie M., A. Tomczyk, J. F. Price and D. Kropczyńska (1988): Interactions between the two spotted spider mite (*Tetranychus urticae* Koch) and leaf miner (*Liriomyza trifolii* Burgess) on chrysanthemum leaves. *Med. Fac. Landbouww. Rijksuniv. Gent, 53/2b,* 811-819.

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THE PHENOL DETOXIFYING ENZYMES OF THE GRAIN APHID

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Key words: Grain aphid, detoxification, enzyme, activity, phenolic compounds, polyphenol oxidase, peroxidase, β-glucosidase, tanase, melanines, glutathione-transferase, winter wheat.

Insects develop physiological-biochemical defense mechanisms which enable them to detoxify secondary plant metabolites. Toxic substances undergo a conversion in the insects. Then the insects excrete these substances or use them to synthesize their own metabolites. The group of detoxifying enzymes comprises hydrolases which are secreted to the alimentary canal and also injected into plant tissues together with the saliva. Intracellular oxidoreductases and transferases also belong to this group (Miles, 1968; Down et al., 1983).

The influence of various concentrations of phenol compounds in a grain aphid's diet (*Sitobion avenae F.*) on polyphenol oxidase, peroxidase activity has been studied. Polyphenol oxidase, peroxidase, β-glycosidase, tanase and glutathione-S-transferase activities in the aphid body were determined. The aphids had been raised on winter wheat with various levels of phenols in its tissues.

Phenol compounds are oxidized by polyphenol oxidase to melanine dies and phlobofenes (Miles, 1968, 1969). Peroxidase takes an active part in these processes. On the other hand, both β-glycosidase, which hydrolyzes phenol glycosides to free phenols, and tanase, which liberates gallic acid from tannin, play a subsidiary role. Then these compounds may be detoxified by polyphenol oxidase and peroxidase. Such a defensive mechanism in grain aphid is possible after ingestion of food. It is also possible in wheat tissues, as enzymes, except tanase, are active in the saliva which the grain aphid injects into the plant tissues (Niraz et al., 1988). It is known that benzoic and cinnamic acid derivatives, and quercetin being toxic to aphids, are oxidized by the grain aphid with the help of saliva enzymes. Peroxidase activity in the body is greater after catechol, or m- and o-coumaric acid absorption. On the other hand, many phenols taken up by the grain aphid reduce its peroxidase activity. No phenol compound has been found to exert an inductive influence on polyphenol oxidase of the grain aphid. All the tested phenols, however, hampered this enzyme activity, acting probably as inhibitors (Table 1).

It has been stated that activities of phenol detoxifying enzymes in grain aphid do not match the phenol level in wheat tissues on which the grain aphid fed (Table 2). Enzyme activities in aphids feeding on the cultivar Saga that is characterized by high

Table 1 Enzyme activity changes of grain aphid fed with phenolic compounds

Enzyme	Trivial compound names	Concentration of compounds		
		$1.0x10^{-4}$M	$2.0x10^{-4}$M	$4.0x10^{-4}$M
Polyphenol oxidase	catechol, gentisic acid, protocatechuic acid, gallic acid, tannic acid, cis-trans caffeic acid, chlorogenic acid, quercetin	activity decreases		
Peroxidase	catechol, o-cumaric acid, m-coumaric acid	activity increases		
	p-hydroxybenzoic acid, gentisic acid, protocatechuic acid, gallic acid, tannic acid, syringic acid, vani	activity decreases		

Table 2 Enzyme activity of grain aphids raised on winter wheat containing different levels of phenols

Enzyme	Variety; phenols content	
	Livilla; low	Saga; high
Polyphenol oxidase A_{460}/30 min	0.28	0.15
Peroxidase A_{430}/30 min	0.16	0.05x
β-glucosidase A_{400}/30 min.	0.25	0.09x
Glutathione S-transferase μmol conjugate/1 min	2.81	6.98

x - value significantly different at $p<0.05$

concentrations of phenols in its tissues, proved to be lower than those in aphids feeding on Liwilla which has a low phenol content. The above results indicate that the grain aphid cannot cope efficiently with phenol compounds present in the food.

Presumably an enzyme important in grain aphid's protection from plant substances is glutathione-S-transferase. This enzyme acts together with microsomal oxidases and also directly fixes toxins, which facilitates cellular circulation and excretion. Females feeding on a resistant wheat variety show higher enzyme level in comparison with a susceptible one (Table 2).

Grain aphid's body does not contain any tanase. Presumably the alkaline reaction of the gut (pH: 7.5-8.3) and of watery saliva (pH: 8.0-8.8) protects insects from tannins as in the alkaline environment tannins present in food do not hamper enzyme activity of insects.

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REFERENCES

Dowd, P. F., C. M. Smith and T. C. Sparks (1983): Detoxification of plant toxins by insects. *Insect Biochem. 13,* 453-468.

Miles, P. W. (1968): Insect secretions in plants. *Ann. Rev. Phytopath. 6,* 137-64.

Miles, P. W. (1969): Interaction of plant phenols and salivary phenolases in the relationship between plants and Hemiptera. *Entomol. exp. appl. 12,* 736-744.

Niraz, S., A. Urbańska, D. Czyżewska and B. Leszczyński (1988): Some physiological and biochemical relations between cereals and aphids. In: F. Sehnal, D. Denlinger, A. Zabza (eds), *Endocrinological Frontiers in Physiological Insect Ecology*. Proc. of the Int. Conf. 7-12 September 1987. Szklarska Poręba, Wrocław Technical University Press, Vol. 1:123-128.

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A COST-BENEFIT ANALYSIS OF THE PYRROLIZIDINE ALKALOIDS OF *SENECIO JACOBAEA*

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Key words: senecionine, *Tyria jacobaeae*, *Longitarsus jacobaeae*, alkaloid content, *Aphis fabae*, *Brachycaudus cardii*.

Previous research showed that *Senecio jacobaea* plants differ greatly in alkaloid concentration. Clones of genotypes reared under identical circumstances maintained their original differences in alkaloid concentration which suggests a genetical basis. Alkaloids are thought to protect the plant against herbivory. If alkaloids protect a plant against herbivory and the alkaloid concentration is genetically determined then selection should favour plants with a high alkaloid concentration. In the field, however, a whole range of alkaloid concentrations is observed in *S. jacobaea* plants. Probably, next to a positive effect, alkaloids also have a negative effect for the plant. Therefore, I carried out a cost-benefit analysis of the pyrrolizidine alkaloids in *S. jacobaea*.

Benefits. In two experimental field populations 900 plants (clones) from 6 genotypes were studied. Number of egg batches of the Cinnabar moth (*Tyria jacobaeae*) and herbivory of the flea beetle, *Longitarsus jacobaeae* were recorded. Differences in number of egg batches deposited by the cinnabar moth and differences in herbivory of the flea beetle between genotypes were highly significant (ANOVA, $p<0.001$). The correlation between the number of egg batches and a particular group of alkaloids (among them senecionine) was significantly negative. No correlation was found between herbivory by the flea beetle and total alkaloid concentration.

Nearest neighbour pairs of flowering plants with and without the aphid *Aphis fabae,* sampled in the field, were cloned in the laboratory. The clones with *A. fabae* had lower alkaloid concentration (Wilcoxon's matched pairs test, $p<0.01$, $N = 35$). Furthermore, a laboratory experiment showed a significant negative correlation ($r = -0.53$, $N = 16$) between the growth rate of the aphid *Brachycaudus cardii* and the alkaloid concentration of the plants on which the aphids were reared.

Costs. We expected that if there were costs involved in alkaloid production a higher alkaloid concentration would lead to a lower growth rate or biomass production. In a hydroculture experiment the correlation between alkaloid concentration and the biomass of rosette plants was indeed significantly negative ($r = -0.55$, $N = 15$). However, another experiment on 11 clones in the greenhouse showed no corre-

lation between alkaloid concentration and percentage flowering or number of flowerheads.

Both field and laboratory studies show that three out of four herbivores avoid or grow less well on plants with high alkaloid concentrations. Costs could be shown in rosette plants but not in stem plants.

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TARSI AND OVIPOSITOR GUSTATORY SENSILLA OF *MARUCA TESTULALIS*: CODING PROPERTIES AND BEHAVIORAL RESPONSES

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Key words: mass rearing, bean, cowpea, oviposition, host-plant resistance, electrophysiology, NaCl, sucrose, legume pod-borer.

Maruca testulalis (Geyer), a tropical legume pod-borer, is a major pest of cowpea *Vigna unguiculata* Walp (Taylor, 1978; Singh and Jackai, 1988). Mass-rearing of *M. testulalis* in the laboratory still relies heavily on eggs deposited on young cowpea plants because this moth is fairly specific in terms of its oviposition requirements. A satisfactory artificial oviposition substrate is needed to facilitate rearing large numbers of *M. testulalis* required for evaluation of host-plant resistance and other studies. Data on oviposition behaviour and stimuli acting on the tarsi and ovipositor gustatory sensilla can be used to make a suitable artificial oviposition substrate. Investigations reported here have concentrated on the following:

(i) Oviposition responses on natural and artificial substrates treated with either known compounds or host-plant extracts.

(ii) Electrophysiological tests to assess the sensitivity and coding properties of the gustatory cells innervating the tarsi and ovipositor contact chemosensilla.

M. testulalis female deposits one or three eggs at any given spot especially on the under surface of the host-plant leaves. Although the common bean plant (*Phaseolus vulgaris*) can serve as an alternative host of *M. testulalis*, a two-way choice oviposition test showed that females laid almost four times more eggs on the cowpea plants than on the bean plants. In addition to this, three-way oviposition choice tests were done using 2-3 weeks old cowpea plants dipped in one of the following: 0.01M NaCl, 0.019M or 0.125M sucrose solutions containing a surfactant Triton X-100 (0.02%). Control plants were dipped in distilled water with the surfactant. The 0.019M sucrose treatment augmented oviposition slightly while 0.125M sucrose reduced the oviposition responses to almost a half of that observed on either the NaCl-treated or the control cowpea plants. This concentration of sucrose apparently inhibited oviposition but served as a phagostimulant because moths spent several hours on the 0.125M sucrose-treated plants licking sugar on the leaves. Filter papers treated with the aqueous extract from fresh cowpea leaves served both as an oviposition and phagostimulant medium while filter papers treated with sucrose alone merely served as phagostimulants.

The ovipositor has twelve robust gustatory sensilla with well defined openings at their tips. Before egg laying, the moth curves its abdomen and drags the extended ovipositor on the filter paper. On the host-plant the same behaviour is manifested but first and foremost a gravid female spends a considerable length of time searching for a suitable oviposition site with its agile ovipositor. In the process the ovipositor gustatory sensilla gather information about the contact chemostimuli on the substrate. The tarsal sensilla provide sensory input leading to the extension of either the proboscis or the ovipositor depending on the prevailing biological urge. A moth presented with a sucrose solution-soaked cotton wool pad, extends its proboscis spontaneously as soon as the tarsal sensilla come in contact with the cotton pad; but this does not happen if the cotton wool pad is soaked with a NaCl solution.

Electrophysiological tests suggest that the response spectra of the tarsal sensilla is broader than that of the ovipositor sensilla. They responded to sugars, cation salts and amino acids. However, among the stimuli tested, the ovipositor sensilla responded to one sugar (sucrose) and cations but not amino acids. It appears that the ovipositor sensilla are more selective than the tarsal sensilla. Furthermore the sensitivity of the two types of sensilla also differ. For example, the sucrose threshold concentration for the tarsal sensilla is 0.019M, while that of the ovipositor sensilla is 0.00118M. Increasing concentration of cation salts evoke responses from one cell in each tarsal gustatory sensillum, but in the ovipositor sensilla similar cation solutions stimulate at least four cells in each sensillum. In the tarsal sensilla, the methanol extracts of cowpea leaves also act on the same cell. On the other hand, sucrose acts on a different cell and results from several recordings indicate that amino acids like proline, glycine and sugars like glucose also act on the sucrose receptor cell. These observations form a basis for further investigations needed to improve certain aspects of the mass-rearing techniques for the legume podborer.

REFERENCES

Singh, S. R. and L. E. N. Jackai (1988): The legume pod-borer, *Maruca testulalis* (Geyer): Past present and future research. *Insect Sci. Applic. 28,* 1-5.

Taylor, T. A. (1978): *Maruca testulalis*: an important pest of tropical grain legumes. In: S. R. Singh, H. F. van Emden and T. A. Taylor (eds), *Pests of Grain Legumes: Ecology and Control.* Academic Press, New York. 195-200.

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TWO-YEAR PERIODICAL CHANGES IN ABUNDANCE OF GALL-FORMING APHIDS: DESCRIPTION AND POSSIBLE CAUSES

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Key words: *Baizongia pistaciae*, Pemphigidae, *Pistacia palaestina*, Anacardiaceae, tree load hypothesis, alternating population hypothesis, population dynamics.

Baizongia pistaciae (L.) makes large galls on *Pistacia palaestina* (Anacardiaceae). Each gall contains the parthenogenetic offspring of a single female. The number of aphids in the gall may exceed 15,000.

Life cycle of *B. pistaciae* is a typical two-year holocycle, alternating between *P. palaestina* (the primary host, on which the gall is formed) and the roots of secondary hosts. Sexual reproduction and gall formation occurs only once in two years.

The density of galls on 50 marked trees has been recorded since 1979 at two sites in Israel. Regular alternation of high and low density was observed on many trees. Time-series analysis of the pattern on individual trees yielded positive autocorrelation between counts 2, 4 and 6 years apart and negative autocorrelations between counts 1, 3 and 5 years apart. At a third site, near the southern limit of the distribution of the host tree, no regular pattern was found on 10 trees. But 4 other trees at that site were burnt to the ground in 1981. Vigorous growth resumed in 1982, and the aphids recolonized the trees in 1983. Population on these trees in subsequent years reached unprecedented sizes, and clear two-year cycles were observed since then.

Two hypotheses are offered to explain the pattern. The "tree load hypothesis" suggests that a heavy load of galls diminishes the accumulation of tree resources and reduces the chance of successful aphid infestation the following year. If the "tree load" hypothesis is correct, trees with a high density of galls should be more clearly periodical than trees with lower densities. This could not be demonstrated.

The "alternating population hypothesis" suggests that aphid populations on odd and even years are genetically different because of the unusual two-year life cycle of the aphids. If the "alternating population hypothesis" is correct, we should be able to find genetic differences between odd - and even-year populations. So far none were discovered.

Conclusion. Ten years of study are too short a time for a real understanding of population dynamics in nature.

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REARING COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA* SAY) LARVAE ON ARTIFICIAL DIET

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Key words: *Leptinotarsa decemlineata*, artificial diet, 17-α-methyl-17-β-hydroxyandrostadien-1,4-on-3, metanabol, chlorogenic acid, survival, phagostimulant.

Rearing the Colorado potato beetle on an artificial diet enables to study the nutritional value of different compounds and to observe the insect's development. Colorado potato beetle is especially difficult to rear on an artificial diet, because it is an insect very selective in food choice. Unusual difficulties appear when the larvae of Colorado beetle on such diet are to be reared.

When artificial diet for phytophagous insects is beeing elaborated, it is necessary to include also host-plant material or some extracts to supply feeding stimulants or trace nutrient compounds as growth factors. The composition of an artificial diet for Colorado potato beetle containing the primary nutrients and secondary plant chemicals was given by Krzymańska and Zwolińska-Sniatałowa (1970), and Zwolińska-Sniatałowa and Krzymańska (1980).

In the following the results of experiments with a diet supplemented with different doses of dried potato leaves as well as chlorogenic acid and metanabol (17-alpha-methyl-17-beta-hydroxyandrostadien-1,4-on-3) improving anabolic processes, is presented.

In the experiments it was found, that the addition of supporting substances as dried potato leaves and some stimulants, especially chlorogenic acid and metanabol, considerably improved the nutritional value of the synthetic diet for Colorado beetle larvae.

Table 1 Development of 3rd instar larvae on different basic diet (BD) combinations

Diet	Percentage of survival of larvae after 6 days	Initial weight of one larva (mg)	Final weight of one larva (mg)	Content of total lipid % in fresh weight
BD + dried potato leaves 20 g	46.15	34.90	32.85	2.19
BD + dried potato leaves 20 g + metanabol 5 g	49.05	35.75	33.30	2.60
BD + dried potato leaves 20 g + chlorogenic acid 5 g	55.60	38.70	46.00	2.90

REFERENCES

Krzymańska, J. and Z. Zwolińska-Sniatałowa (1970): Elaboration of basic artificial diet composition for Colorado potato beetle. *Bull. Acad. Pol. Biol. 18,* 785-787.

Zwolińska-Sniatałowa, Z. and J. Krzymańska (1980): The development of the larvae of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) reared on a modified artificial diet supplemented with some growth stimulants. *Bull. Acad. Pol. Biol. 28,* 785-787.

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GENERAL CONCLUSIONS

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The initial inspiration for the Insect-Host Plant Symposia came from a section at the International Congress of Entomology in 1951 which was entitled "The physiological relations between insects and their host plants", and the emphasis at the earlier symposia was indeed physiology and behavior, especially as they relate to host plant selection and crop protection. Since then the scope has rapidly increased to the situation at this symposium, where for the first time exactly half of the presentations in lectures and posters relate to ecology and evolutionary biology. The types of interaction are many and varied and the diverse array of work has been particularly rewarding for interactions among disciplines: ecologists have become involved in physiological and behavioral mechanisms; behaviorists and physiologists want the ecological perspective; causal and functional mechanisms are of equal interest. Where ten years ago it was seen as a real achievement to combine chemistry and entomology it has here been commonplace to combine chemistry, biochemistry, physiology, genetics, behavior and ecology. Above all, the evolutionary aspects of relationships have become prominent. It is a very exciting time because the barriers between disciplines really are breaking down and we see each idea or data set becoming a fertile seed for someone in a different field.

Such a mixture is certainly invigorating; it is also overwhelming. Is it possible to crystallize something from so much material as well as the infinite number of small details and fleeting moments that make up a fruitful meeting? This concluding section is a personal selection of points. Such selections inevitably depend on those moments of intensity in our rhythms of attention and on associations that are ready to be made, but perhaps they help distill the essence that will linger, and become *aides mémoires* for the rest.

It is appropriate to begin with the subjects that have been important since the symposia began: why insects eat what they eat. At the proximate level we have seen considerable progress. The chemoreceptor codes have seemed so complex and variable but there is light at the end of the tunnel. At least three kinds of codes appear to operate, and the combination of peripheral and central nervous recording

promises to reveal before long, what has been so elusive. The chemicals that stimulate and deter feeding are ever changing in the plant, and the cells that respond in insects vary in their thresholds with the ever changing physiological feedbacks. The whole is more finely tuned than we thought.

There are important repercussions for the study of behavior. We need to examine individuals for long periods or we miss the important details and differences in the noise created by our hurry. So it has been demonstrated that insects select food in ways that precisely compensate for deficiencies. It has become clear that the level of sophistication in nutrient feedbacks is equal to that in any animal and it may even be that it is in caterpillars and grasshoppers that a complete understanding of nutrient feedbacks is found first.

The closer look has highlighted the importance of multiple sensory modalities in behavior and their synergism in host acceptance. The plant surface has been shown to be ever more important both physically and chemically. More detailed studies, with these facts in mind will take us further, more quickly, in understanding and improving crop resistance and natural enemy success.

Field behavior is demonstrating intricate differences among and between species that we can better exploit. Learning has been shown to be widespread but of variable importance for the individual. If experience can influence host plant finding in the field, perhaps it will prove to be important as a component of increased pest incidence in large monocultures.

The growth curve of work on the plant chemicals involved in host attraction, acceptance or rejection slowed a little, but important progress has been made. Less well known chemical groups are proving to be just as important as the famous ones. New techniques with phloem feeders have allowed investigation of what they really taste. Toxins may become deterrents after ingestion. Deterrents and repellants seem more definitely to dominate, though many of them are harmless.

The evolutionary determinants of host selectivity and host affiliation are more elusive. We can be sure that the first factor is availability with all its components including patch size and temporal constancy. There are presumed phylogenetic constraints - scale insects tend to be polyphagous yet aphids to be specialized. Do the differences represent ancient speciation patterns vs. more recent ones? When host use is already narrow perhaps simple changes in the chemoreceptor system or the profile of chemicals in plants can lead to a change in host fidelity. Perhaps other trophic levels provide important selection on host range. Are specialists favored under pressure from generalist predators and pathogens? On the other hand, do specialist parasitoids select for host shifts or polyphagy? Are specialists associated with early successional stages? Clearly there are many answers and we are a long way from a synthesis.

Coevolutionary hypotheses have been viewed with scepticism in the light of the commonly underestimated level of stochastic events in nature. On the other hand we have the demonstration of a closely interacting plant and herbivore each genetically varying in relevant characteristics. It is possible now to have agreement that in such a system there can be close reciprocity in the evolutionary process. Much that

was combative has fizzled into semantics, for clearly multiple selection pressure exist and they alter over time, chance events alter processes and adaptation need not be perfect but simply good enough. It may help to let the "co" go.

An important requirement in reciprocally adapting insect-plant systems is that insects have impact on their hosts. There are examples of different kinds of impact and expressions of doubt as to its significance: more work is essential on the levels of damage and how this relates to fitness of plant. Some insect species have an effect and many do not. Input from plant ecologists is needed. New ideas from plant molecular genetics indicates that the effect of environmental factors can influence regulatory genes in plants during somatic growth. The resultant changes give rise to two important things in the discussion of insect-plant evolution. Firstly, extreme within-plant mosaics are very common and secondly, there can be preservation of these changes in gamete production. Both of these could lead to greater potential for plant changes that effect herbivores in ecological and evolutionary time scales.

Ecological patterns have been presented that have a greater evolutionary perspective than ever before. Do rare species of phytophagous insects have some distinctive constraints or adaptations that contrast with abundant or eruptive ones? Contrasts seem to exist for tree feeders at least. Theories of plant defense have been combined in a new framework with a combination of ecological and evolutionary elements providing new stimuli for proof and disproof. Changes induced in plants by herbivores have been a recurring theme: they are many and varied. Are they specifically adaptive in relation to herbivory - detrimental to the herbivores or attractive to their predators? Or are the changes essentially physiological changes related to wound healing and regaining biochemical balance, and incidentally protective through being different and deterrent, while natural enemies adapted to the chemical signal of prey presence?

The continued synergism of combining studies of proximate and ultimate causes, and interacting in meetings like these, must be the way forward. At several levels input from plant physiologists and plant ecologists is wanting. There is a still more urgent need for genetics and molecular biology. In the 1978 symposium the absence of genetics was bemoaned and the need has become more pressing. Although quantitative genetics is under way in labs not represented here, and there have been several important glimpses of genetic work at this meeting, much more is possible. For example the use of mutants with chemoreceptor abnormalities could be invaluable in developing an understanding of host recognition by hervivores. Molecular biology has even more for the future. A common response of entomologists to an increase in funding for biotechnology is annoyance and frustration and an increasing distance from it. Collaboration, on the other hand, would be intellectually and financially profitable. Determination of relationships and divergence times is within easy reach, making gene probes for chemoreceptors that differ in two populations is possible. The effect of a single gene change may give an idea of how readily this could lead to a change in host preference. Genes for single enzymes can be implanted in plants, genes for regulatory hormones can be delivered to insects. The possibilities for the future are almost certain to be greater than we presently imagine.

Finally I want to pay tribute especially to Tibor Jermy. Over the years he has so quietly but profoundly influenced the field of plant-insect interactions. He has been a leader in many aspects of behavior as well as in its application. He has been the one who gently questions our conclusions and our theories. And he is proving often to be closer to the truth than we first believed. We dedicate this symposium and the printed volume to him.

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