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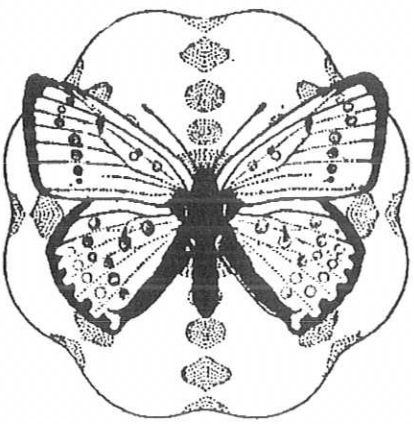

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THIS VOLUME CONTAINS THE PROCEEDINGS OF THE FOURTH MEETING OF EXPERIMENTAL AND APPLIED ENTOMOLOGISTS IN THE NETHERLANDS

Ede, 18 December 1992

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EDITORIAL

Experimental and applied entomological research in the Netherlands covers a wide range of different aspects. The aim of this annual proceedings of the Netherlands Entomological Society is to provide a recent overview of this research. It is an important objective that this series serves as a fast publication medium. We therefore intend to have the manuscripts published within a few months after submission. All authors are provided with 50 reprints of their contributions.

To improve the scientific impact of this publication, steps were undertaken to have this series covered in a number of review journals. It is currently being listed in the databases of the Institute for Scientific Information (including "Current Contents") and of the Cambridge Scientific Abstracts (Entomology Abstracts / Animal Behavior Abstracts). Furthermore the number of copies of this edition was increased in order to enhance the distribution of this series especially to scientific libraries. The cooperation of Mrs. I.I.Riphagen, Utrecht University Library, is appreciated in this respect.

We are pleased with the steadily increasing interest of the different entomological institutes and specialists in the Netherlands to contribute to this publication.

January 1993

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PREFACE

18 December 1992 for the fourth time the annual "Nederlandse Entomologendag" took place, organised for and with the experimentally and/or applied working entomologists in The Netherlands.

As in 1991 the Meeting was held in Ede and organised in collaboration with the Wageningen Agricultural University (WAU).

About 200 participants attended the meeting, covering all the disciplines within entomology on which research is done in The Netherlands. Forty-five papers and six posters were presented, most of them published in this Proceedings. For the organising committee it is very rewarding to experience such an enthusiastic and numerous participation.

We hope the multi-disciplinary supply of subjects discussed during the Meeting will be an impulse to cooperate together in interdisciplinary research programmes. It will be the only way to unravel the high complexity of biological (including entomological!) problems.

Sandrine A. Ulenberg
chairman of the section
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INSECT-PLANT RELATIONSHIPS: ATTACK AND DEFENSE IN TIME AND SPACE

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Key words:
allomones, co-evolution, herbivores, natural enemies, phytophagous insects

Summary

During evolution both plants and insects have developed intriguing strategies to exploit each other at acceptable risk levels. About half of the world's insect species are associated with plants. The relations may seem to be very useful and peaceful, but in fact there has been a continuous war without armistice, during which defense systems were developed to be broken by adaptations of herbivores. To that end phytophagous insects have found different solutions.

Many thousands of plants and insect species are in a dynamic equilibrium. Plants have to defend themselves against much more than phytophagous insects alone, and host-plant resistance to a particular herbivore is often partial. For terrestrial plants natural enemies of herbivores form a protective umbrella; the combination of plant defense and bioregulators keeps our world green.

Domestication of wild plants to highly productive crops results in considerable loss of host-plant resistance. An increased resistance based upon a specific compound toxic to insects can be expected to be short-lived, as it enhances selection pressure towards more agressive insect biotypes.

The best possible plant protection can be reached in an agrotechnical system which integrates habitat quality and host-plant resistance. Mineral nutrition of the plants, repellents, deterrents, partial host-plant resistance and optimal activity of natural enemies can be combined to arrive at an environmentally safe crop protection system.

INTRODUCTION

There is enough food on the world for all human beings, and still many people are dying of starvation. At present this problem can be solved technically, but human kind is unable to cope with it because of incomplete socio-political evolution. There would be no misery on our planet when populated by a limited number of *Homo sapiens*, optimally exchanging goods and constructive thoughts.

Biologists use to speak of the Plant and Animal Kingdom, a description that renders us to presume the existance of a secure and perhaps better world than the artificial environment in which we find ourselves - and yet the two are closely related.

Little more than basic knowledge of evolution and population dynamics is needed

to understand that for individuals of whatever form of life this planet is no Paradise and that there is hardly a species, however ingenious its direction of development, that is not threaded with irradication by other members of the living world.

Being a herbivore is just another way of struggling along a weary way winding between trophic levels below and above, haunted by natural enemies and condemned to feed upon plants that are difficult to trace, often nutritionally suboptimal, or full of poisons.

Roughly speaking a bit more than half the world's insects are either parasitoids or predators or feed on dead organic material, leaving a substantial amount of phytophagous insects. There must be some degree of success in a herbivorous life and a close look to even a mighty tree in a forest learns that it stayed alive with more than a fair share of the worlds biota: from the roots up to the tiniest leaves on top, insects gnaw and suck with an amazing diversity. Cohorts of insects feed invisibly upon the array of roots; ants dig their nests deep into the trunk; wood-piercing beetles seek for weak sites in the bark to lay their eggs, and later their larvae will tunnel through the wood. Leaf miners live in the thin layer between the upper and lower epidermis of the leaves, caterpillars gnaw at ease; aphids and tree-hoppers drain the sap with their sucking mouthparts. Still, the tree is there and the world around us is green, demonstrating that both plants and insects have developed strategies for survival.

The relationship between plants and insects is of inimagable complexity. During their larval stages insects can almost destroy a forest to become pollinators as adults resulting in seed production for a new generation of seedlings. Plants can also "eat" insects, be it after trapping with glandular hairs as in *Drosera* spp., or catching the insects in a mortal dungeon, as in beaker plants. As may be expected, there is always a further step - some insect species actually live in these traps, predating on catched insects or laying their eggs deep in the dangerous pit, insensitive to the digestive enzymes.

WHY DO INSECTS SELECT PARTICULAR PLANTS?

One of the best studied relationships between plants and insects, of course, is that of the pollinators. The mutual service of this relationship seems to speak for itself - yet it is the result of a long-lasting war between two different forms of life, but only a minute of the astronomical time scale - angiosperms developed from about the beginning of the cretaecious period.

Phytophagous insects date from a much more archaic time and tend to specialize on certain plant families, -species, or even parts. Why do they specialize and why is the majority of species never abundant? It is not the occasional spectacular outbreak, but the enormous diversity of plant-insect combinations that make phytophagous insects so interesting.

Already the opening address of the 7th International Symposium on Insect-Plant Relationships in Hungary, 1989 (Schoonhoven, 1991) states that insect-plant relationships as we can study them today, are not in a static condition, but show an interaction between two very complex forms of life. This relation would seem to be characterized by an enormous chemical diversity of the Plant Kingdom and appropriate readjustments of the chemosensory systems of insects. Does this vision reflex

reality?

A number of biological reasons can be presented to explain host specialization:
- plant chemistry
- availability of specific nutrients
- risk avoidance (physical factors + natural enemies)
- necessity of mate finding
- selection of the best hosts by chemical clues.

Even when only one of these items may cause host specialization the final relationship can be reached by:
- co-evolution
- subsequent evolution
- non-adaptation to allomones (toxicity of non-host chemicals is the result and not the cause of specialization)
- food-limitation.

Most of the work on insect-plant relations cover the angiosperms, and to get some understanding of the development of these relations it is of interest to see what we know of older plant taxa. Ferns are the most ancient (of extant) groups of widely distributed vascular plants and obviously very successful, as they are only surpassed in species diversity by the angiosperms. Ferns are recorded from the late Devonian. Insects had a considerable development already in the Carbonian. When we compare the ratio phytophagous insects to plant species there seems to be a great difference (Cooper-Driver, 1978) (Table 1). There have always been insects on ferns, and the fossil recordings are mainly these of Coleoptera and Hemiptera, whereas today the majority of phytophagous insects on ferns belong to Hemiptera, Coleoptera and Lepidoptera.

Table 1. Relationship of phytophagous insects with angiosperms and ferns

	Nr of species	Ratio insects/plants
Angiospermic plants	286×10^3	
		1:0.8
Associated phytophagous insects	358×10^3	
Ferns	223×10^3	
		1:24
Associated phytophagous insects	9.3×10^3	

The ratios suggest that ferns are less used by insects as angiosperms, although ferns may be preferred above other plant taxa in certain regio's (Balich *et al.*, 1978). A bit less than half of the insects on ferns are sap feeders and seem to thrive on the phloem. Has this relationship been established because ferns did not develop an active defense system against phloem-feeders? Homoptera, among which aphids, have the widest spectrum with regard to the species of Filicinae. Coleoptera do not

Table 2. Main theories of plant chemical defense

Theory	Main features	Insects that can use the plants as a host
(1) Tissue value hypothesis	"Costs" of defense in relation to necessity of protection	
	High levels of protection (generative parts). Defense to generalists	Adapted specialists to feed on fruits, pollen etc. Well-developed detoxification mechanisms
	Low level of protection	Generalists, N-dependent species
(2) Apparency hypothesis	Defense dependent on traceability of plants. Inapparent - qualitative resistance. Defense to generalists	Adapted to carbon-based defenses. Detoxification of related allomones
	Apparent - quantitative resistance. Defense to specialists	Adapted to poor nutrition, anti-digestives, and feeding site. Use of kairomones
(3) Resource availability hypothesis	Resources in the habitat determine degree and type of defense	
	Fast growth: highly active, mobile and specific compounds at low concentrations	N-dependent species that can detoxify alkaloids and cyanogenic glucosides
	Slow growth: high levels of defense	Adapted to toxic nutrients, N-independent species. Phloem feeders
(4) Habitat templet model	Integration of (2) and (3). Apparent plants in unfavourable habitats: high levels of defense	Adapted to very toxic compounds. Use of kairomones. High energy-efficiency
	Inapparent plants in favourable habitats: low level of qualitative defense	Well-developed anemotaxis. Sensitive to volatiles. Effective sex pheromones. Short generation time

feed on phloem and almost half of the species on ferns are found on the big tree ferns of the *Cyatheaceae*.

It is tempting to compare these facts with the theories generally applied to

explain plant defense, as surveyed by Feeny (1991) and presented in condensed form in Table 2.

SPECIALISTS AND GENERALISTS

Although it is stated in the Introduction that herbivores tend to specialize, Table 2 uses the terms specialists and generalists. This generally accepted terminology is somewhat misleading: even generalists do not thrive on many plant taxa, but may accept up to a few hundred of host species. Specialists are oligophagous or monophagous. What defense systems do ferns have? They lack alkaloids and glucosinolates, both a speciality of angiosperms.

Alkaloids are very toxic to mammals, but mammals were absent during times ferns had to defend themselves already to insects. Ferns seem to produce both qualitative compounds, such as terpenoids, non-protein amino acids and phenolics, especially flavonoids; they also contain quantitative compounds, of which condensed tannins are well-studied. It would seem that the use of different defense strategies is not novel and has been invented already in ancient times. This also holds for antixenosis versus antibiosis, to use this unsatisfactory distinction between allomones: sesquiterpenes act as feeding deterrents and the enzyme thianiase that causes vitamin B_1 deficiency is a toxic compound, to give a few examples.

SPECIALIZATION ON FEEDING SITE

The high proportion of phloem-feeders among fern insects indicate that other tissues are no easy food. According to Table 2 tree ferns should resort under quantitative resistance and are mainly associated with Coleoptera. Today, tannin in higher quantities is still effective upon beetles, although low doses can mitigate the toxic effects of other allomones, such as cyanogenic glycosides (Goldstein, 1987).

Tree ferns certainly contain tannins, but they could be there for other reasons than defense to insects. Unfortunately, most of our knowledge of tannins in ferns is on *Pteridium* spp., and the facts available do not speak for a well-defined correlation between tannin content and insect resistance (Bernays, 1978; Cooper-Driver, 1978). Moreover, *Pteridium* belongs to the Polypodiaceae that are most commonly attacked by insects.

Still, the *Filicinae* have proved to be a very successful class of the Pteridophytae, armed with a modest diversity of protecting metabolites, and able to cope with herbivores of which about 70% are specialists restricted to ferns.

In comparing the ancient Pteridophyta with the Spermatophyta, the seed-producing plants, the Gymnospermae, although more primitive than the angiospermae, prove to have a very successful design with plants ranging from a fern-like plants to enormous trees among the Coniferae. The end of Perm and Triassic brought a rich development and omnipresence, especially of the Cupressaceae.

Gymnospermae have their deal of insects, but as almost all species spread their pollen with the wind, development of a specific relationship with insects as happened with angiosperms, did not occur. It is, however, not easy to find many well-documented studies of defense in the relationships of Gymnospermae with insects.

DEFENSE SYSTEMS

Many terpenoids contribute to the odour of conifers and increased release of odour by rupture of resin-containing tissues, as will happen in wind-damaged trees would make pine trees easy to find by the pine shoot beetle, *Tonicus piniperda* (Schlyter & Löfqvist, 1990). (R)- and (S)-α-pinene and terpinolene are supposed to the have a role as kairomones. Kairomones, by origin, were once allomones and the beetles are thought to react positively to the terpenoids because the host can then be colonized before other species can compete.

High concentrations of terpenes in conifer needles are reported to reduce size and fecundity of spruce budworms (Tortricidae) (Redak & Cates, 1984) and although high terpene content could account for the existence of resistant strains of Douglas fir, there is no consensus with respect to the effectivity of terpenes (Sanders, 1991). Pungenin, for instance, has been identified as a feeding deterrent, but *Picea pungens* belonging to spruces with the highest concentration, is fed on by spruce budworms without any difficulty.

Many tortricid pests of conifers have been described, but control usually relies on insecticides (DDT still being used) or pheromones, occasionally biological control with natural enemies or pathogens such as *Pseudomonas* spores.

Host-plant resistance based on allomones has not obtained much attention, except for North-America, maybe because little is known about tropical tortricids of great importance. A general picture arises: tortricids can severally damage conifers over great areas, but although in a bad shape, the trees often survive. Spruces and Douglas fir survive by carrying large amounts of foliage and by producing adventive needles in response to defoliation. In this way chemical defense is less important against specialist feeders.

FROM GENERALIST TO SPECIALIST

Gymnospermae are attacked by many species of amored scale insects, most of which are oligophagous, although many phytophagous species also appear frequently on conifers. For some scale insects conifers are just a few of a great number of possible hosts. An example is the Latania scale, *Hemiberlesia lataniae* that is known on hundreds of host plants: it also appears on Cupressaceae and Pinaceae. Some species are considered as very noxious: the juniper scale *Carulespis juniperi* can virtually kill entire trees in the United States (Johnson & Lyon, 1776). Unfortunately, little is known of chemical defense of conifers to scale insects, that makes it difficult to place them somewhere in Table 2. This may be partly caused by a number of unsolved questions related to their physiology of feeding. Armoured scale insects do not produce honeydew, as aphids do, and may return surplus materials and metabolites to the plant via the large salivary glands (Banks, 1990). The Diaspididae may have evolved from the lower Cretaceous and can be regarded as a young group that co-evolved with the angiosperms (Danzig, 1980) on which they are mostly polyphagous. This would mean that they have not primarily evolved along with the Gymnospermae on which they are less polyphagous, again with exceptions, such as the Japanese diaspidid *Fiorinia externa* that now colonizes 96% of the conifer species in Connecticut.

"SPECIAL" SPECIALISTS - APHIDS

The first aphids appeared in the Late Permian or in the Triassic, maybe in connection with the evolution of the gymnosperms. In the early days they were not yet heteroceous. Many "modern" families came into existence in the Cretaceous and this change could be connected with the transition from the era of gymnosperms to that of angiosperms, although a large part of the Early Tertiary aphids were probably still associated with gymnosperms, and their relatives still are today (Heie, 1987).

A comparison of the specificity of East European aphids learns that on conifers aphid species are confined to one host species, or at least a group of closely related species. On horsetails and ferns this figure is only 25% and here the aphids are much more generalistic. This may be because in contrast to one would think, aphids on Equisetaceae and ferns belong to very recent genera.

Many "modern" aphid species show host alternation: the eggs overwinter on one plant species or at most on a small group of hosts that are woody. Next spring winged aphids fly to botanically non-related plants. After a number of generations on these secondary host plants winged sexual morphs return to their original, primary host plants. The secondary host plant may belong to different plant families (Hille Ris Lambers, 1979), as shown in Table 3.

Table 3. Secondary host plants of aphids with apple as the only primary host

Aphid species	Sec. host plant	Plant family
Allocotaphis quaestionis	*Senecio* spp.	Compositae
Dysaphis anthrisci	*Anthriscus silvestris*	Umbelliferae
Dysaphis brancoi	*Valeriana* spp.	Valerianaceae
Dysaphis chaerophylli	*Chaerophyllum* spp.	Umbelliferae
Dysaphis plantaginea	*Plantago* spp.	Plantaginaceae
Dysaphis radicola	*Rumex* spp.	Polygonaceae
Ovatus insitus	*Lycopus* spp.	Labiatae
Rhopalosiphum insertum	grasses and cereals	Gramineae

The primary host plant is the same for all aphid species (*Pyrus malus*), but only two species have one secundary host in common. At a closer look, these host species although present in more than one plant community, can be found in the same habitat, such as the Fluviatile district. It would seem that there is a tendency for aphids to exploit abundant species of plants growing in the same or adjoining habitats (Hille Ris Lambers, 1979). Initially the host range of aphids may have been relatively wide, but subspecies developed, each specializing on certain host plants within the total host range.

Most aphid species find their hosts more or less by accident: they have a poor vision, are easily blown by the wind and therefore can not reach a plant even if they could select it from a distance. Host plant acceptation, therefore, mainly results from anatomical and chemical properties of the plants. Phloem feeding led to parallel

evolution between plants and aphids, but their way of life leading to exploit abundant plant species resulted in frequent acquisition of newly developed plants. In other words: There is substantial evidence that aphids adapted to the quality of new host plants rather than shaping their evolution. As there are relatively few plant species that are sufficiently common more oligophagous and monophagous aphid species are associated with common than with plant species that are scattered in the landscape or rare. These plant species have escaped in space.

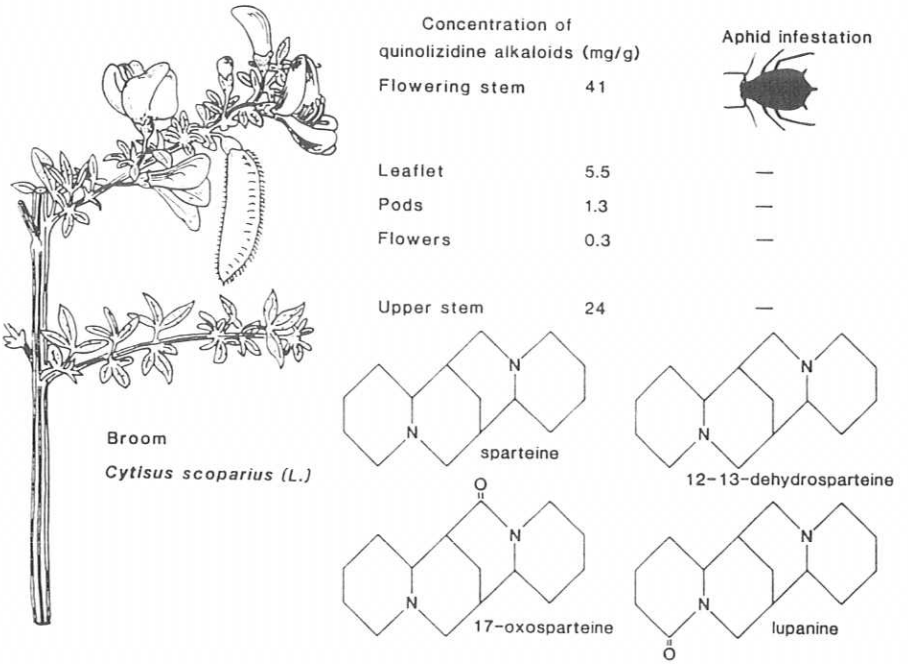

Fig. 1. The total concentration and distribution of the quinolizidine alkaloids, sparteine, 12-13-dehydrosparteine, 17-oxosparteine and lupanine in broom in relation to aphid infestation (After Wink et al., *1982).*

In general, host alternating aphid species are much more polyphagous than monoecious ones. The secondary hosts exploited by aphids are herbaceous plants, often annuals, and are liable to dramatic changes in density, while the woody plants on which most of the monoecious aphid species live, have far more stable population structures. As Table 2 shows, aphids that can detoxify specific allomones, should prefer plants with qualitative defense, as long as they are apparent enough for their limited search behaviour. With respect to host orientation aphids have a rather specific position among herbivores. As MacKenzie & Dixon (1991) state: "In specialization on a constant resource (the woody primary host) and diversification over a range of stochastically varying herbaceous species, the heteroecious aphid species has the best of both worlds". It should be noted that even generalistic aphids

have a limited host plant range with a few exceptions (*Myzus persicae* has more than 300 secondary hosts), but most of them have specialized on a particular host plant range in a certain habitat. Specialized aphids do not avoid nasty allomones, that can even turn into kairomones. A few examples are *Brevicoryne brassicae* that goes for cabbage and likes mustard oil glucosides such as sinigrin, and *Aphis cytisorum* that prefers to feed on the flowery stems of broom, which contains more quinolizidine alkaloids than other parts of the plant (Wink *et al.*, 1982).

FROM GYMNOSPERMS TO ANGIOSPERMS

Speciation of angiosperms is governed by many environmental factors, and the evolutionary stress caused by herbivores is only part of them. The enormous array of compounds against fungi and microorganisms already present or induced (phytoalexins) as a defense against pathogens learns us that plants have to cope with a greater diversity of organisms than writers of horror stories can think of. Aphids are careful feeders and apart from a few exceptional cases, seldom kill their hosts. Therefore, Aphididae may have followed rather than directed the evolution of their hosts. Studies of plant-sucking bugs such as mirids and coreids have revealed that they can remove the contents of cells without mechanical damage (Miles, 1987). Most sap-sucking insects are small in contrast to leaf-eating ones: caterpillars and locusts can completely defoliate a region.

THE IMPACT OF INSECTS ON PLANTS

Did insects stimulate the enormous variation of alkaloids, terpenoids, phenolics to mention alone some of the "qualitative" defense compounds? If so, there should be an evolutionary advantage to develop them specifically against insects and let evolution presure select those that are effective against pathogens and herbivores. Some of the toxins will interfere with normal plant cellular processes unless stored in a bound form, for instance, a glycoside. The toxic substance is liberated upon penetration of the host. *Hedera* saponins are an example. When ivy leaves are damaged by penetration of a parasite, they are partly hydrolyzed, forming α- and ß-hederin, both highly toxic to fungi (Fig. 2a). Many defense substances are known to be synthesized upon attack of a pathogen, ranging from simple phenolic derivatives, such as benzoic acid in apples, to complicated molecules such as gossypol in cotton (Fig. 2b).

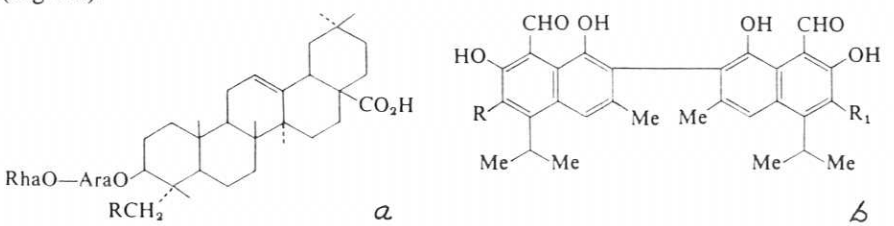

Fig. 2. The fungitoxic allomones Hederin (a) and gossypol (b) that both act as a qualitative defense to insects.

Both types of defense substances can be effective against phytophagous insects as well. Sinigrin, known from crucifers, has fungitoxic properties (*e.g.* against *Peronospora parasitica* in cabbage), and is a deterrent for some generalistic herbivores. This compound has been subjected to quite different functions: it has also been the first host-specific chemical described as a feeding stimulant for specialized insects like *Pieris brassicae* (Verschaffelt, 1910) in a time that the terms allomones and kairomones were not yet introduced.

Many of the secondary plant substances, whether they act as antibiotics, fungistatics, on anti-feedants and toxins against herbivores, represent diverse chemical groups, often associated with plant families. Isoflavones, for instance, are common among Leguminosae, and terpenoids will be found in a number of families depending on their complexity: monoterpenes often as volatiles in essential oils, diterpenoids in latex and resins ("quantitative" defense, Fig. 3) and toxic cardenoides in Apocynaceae, Asclepidaceae and Scrophulariaceae. Solanaceae also have a number of defensive terpenoids.

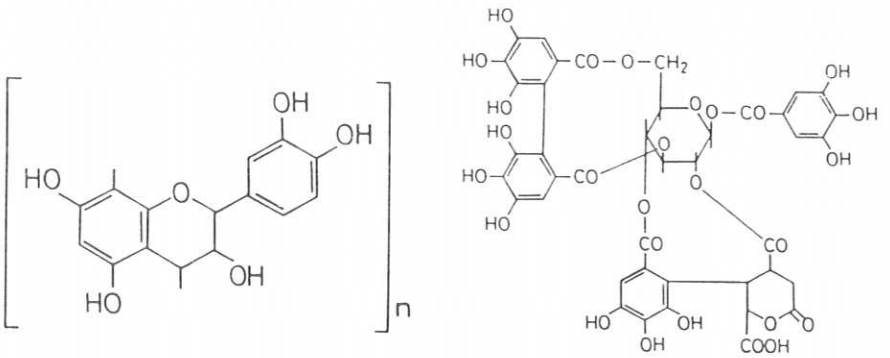

Fig. 3. Quantitative defense: condensed tannins (procyanidin, a) and hydrolysable tannins (chebulagic acid, b).

When these classes of secondary plant substances had been developed against specific insect taxa, one could expect a subsequent relationship with specialistic herbivores, associated with particular plant families. Table 2 has already shown that these relations are much more complicated. Except for nutritional requirements, is that because insects will accept their hosts because of a specific phagostimulant (like Verschaffelt's documentation) or ratio of some, or is host plant specificity mainly determined by plant substances inhibiting feeding? One should consider that remarkedly few feeding stimulants have been discovered and that a wealth of feeding inhibitors are already known. Jermy (1991) is strongly in favour of the latter theory and states that correlation between plant and insect taxa often seems to be better than it is and gives explanation of closely related species or even subspecies that thrive on a totally different plant genus. He argued that the evolution of plants directs that of phytophagous insects by its impact on the selection of mutants. In this respect it should be noted that Bernays (1991) is convinced that selection based upon secondary substances alone is a simplification.

IMPORTANCE OF NATURAL ENEMIES

The generally accepted opinion that selection is based on allomones should at least be supplemented by the view that generalist natural enemies provide a selection pressure for a narrow host range. Bernays is not the only one to believe that the impact of insects on plants is primarily regulated by natural enemies (Lawton & McNeill, 1979). As natural enemies may both be attracted by their prey or by the plants they are on, it is not surprising that plants can produce semiochemicals guiding their protectors towards the herbivores (Dicke *et al.*, 1990). The above mentioned facts indicate that toxicity of non-host chemicals to specialist insects is the result and not the cause of specialization. This would mean that specialist insect species have lost the ability to cope with secondary plant substances that do not occur in their hosts. This seems to be the sequence of events in the case of various groups of graminivorous grasshoppers whose derivation comes from highly tolerant polyphages (Bernays, 1991).

DEFENSIVE POWER OF ALLOMONES

The question rises: How toxic or at least effective are allomones to insects? Anyone acquisited with pharmacy knows that plant-derived medicines are mainly based upon products of angiosperms. Many of them are alkaloids and terpenoids, already emperically used for centuries, including those that affect the human mind. However, it would be questionable to state that plants developed psychopharmaca (*e.g.*, lysergic acid, lyosciamine, atropine, scopolanine) to make herbivores more happy! Obviously, the compounds are there for other reasons (Fig. 4).

Many allomones, especially alkaloids, cyanogenic glycosides and terpenoids, are very toxic to mammals. To generalistic phytophagous insects, however, alkaloids, cyanogenic glycosides and glycosinolates are on average two orders of magnitude less toxic. For terpenoids this factor could be about one order. Phenols are more difficult to compare. They have many functions and are often tested in artificial diets, reducing food quality because of their reactivity (Bernays, 1982).

Nevertheless, phenolics such as caffeic and chlorogenic acid are supposed to have a protective role in cabbage against *Pieris* caterpillars (Van Loon, 1988).

On the other hand, phenols, that are synthesized from aromatic amino acids, can be used by insects, especially by tree feeders when protein concentration is low. It is even essential in the diet of *Bombix mori* (Kato, 1978).

Why are insects relative insensitive to toxic substances? Both anatomy and phenology differ from vertebrates:

- Insects have peritrophic membranes that can selectively absorb macromolecules and these membranes can be substantial in polyphagous species.
- The midgut epithelium has a high potential for detoxifying enzymes, such as mixed function oxidases. This means that detoxification starts before the toxins are released into the haemolymph, which is a more strategic site than the vertebrate liver.
- The insect blood/brain barrier is particularly effective, protecting the central nervous system to a high degree.
- The heart is very simple, often less vital, and phytophagous insects have developed

an additional low sensitivity to cardiac glycosides.
- Insects are poikilotherm. The energy expended to maintain body functions is low in insects, even orders of magnitude less than in mammals.

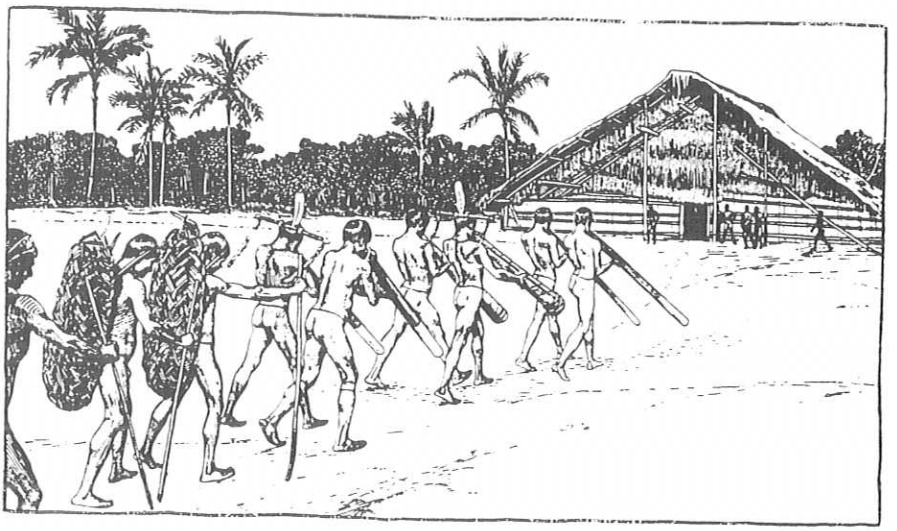

Fig. 4. In the confines of Brazilia natives of the Turkano tribe prepare themselves to speak with the death after intake of the drug tetrahydrochamin, an alkaloid from Banisteropsis *spp. and also present in* Peganum harmala. *Qualitative defense or a present of the Gods to the Indians?*

This list could suggest that insects are particularly successful in avoiding allomones, and may have succeeded to adapt themselves to nothing more serious than their use as kairomones. The many volatile monoterpenes that direct pollinators to their hosts, would speak for such a relationship. Indeed, angiosperms shifted from pollinating mechanisms depending on physical factors to transport by biotic factors, mainly insects, that were lured to the flowers by aromatic compounds, to be rewarded by eadible parts or nectar. A variety of volatiles that can be incorporated in oils, are effective antibiotics indicating that they are not only produced to please or even deter insects. The array of pleasant types of honey on the shelves of many shops let us believe that at least the evolution of bees has been in close harmony to that of the angiosperms. However, a pollen digestion study of Velthuis (1991) shows us that this relationship is far from harmonious. The literature on pollinators proved interesting examples of adaptation of the insects and counteradaptation of the plant (Barth, 1985), in which the plants have to protect the reproductive organs from being destroyed altogether.

When insects are difficult to intoxify, why then try to keep them away with allomones? This would seem to be a gap in our knowledge, but there are a few factors in insects that may help to answer this question.

First, phytophagous insects may be poikilothermic and need less fuel than vertebrates, but they need a far more higher protein level in their food than mam-

mals. The protein to carbohydrate ratio is somewhere between 1:1 and 1:3, whereas in mammals it is an average of 1:8. Population development of polyphagous insects is fully dependant on high protein sources. Young plant parts and developing fruits are rich in protein, and these tissues generally have the highest levels of toxic secundary metabolites.

Second, proteins themselves cannot be traced from a distance. Amino acids can act as feeding stimulants, but their local presence is not necessarily related to host plant quality. We do not know whether "green leaf volatiles" could signal a protein-rich host. When insects use allomones to that end, they have to turn them into kairomones first.

Further, plants are endowed with a variety of defense systems. As can be derived from Table 2 we can make an (as yet incomplete) list of possibilities:

MULTIPLE DEFENSE SYSTEMS IN PLANTS

- Being difficult to find, (Table 2, less apparent). Plants cannot see and are immobile, but can be positioned in the landscape in such a way that they are easily overlooked. Dixon (1987) uses this item to explain why there are so few aphid species in the tropics.
- Mechanical barriers. Thorns, needles, waxes and other epidermal structures. Less effective against small sap-sucking herbivores.
- Optimal protection of reproductive organs. This is already mentioned and can be further improved by unpredictable dispersion of seeds securing persistence in the habitat. Perennial, predictable plants may be subjected to a greater feeding pressure (by specialists) than more emphemeral plants, but could afford a higher metabolitic price for defense. A short developmental period for the reproductive organs is of advantage. They are short-lived resources only useful to insects with a rapid development. Grasses have a vegetation point close to the soil and can be substantially grazed without being destroyed.
- Imbalance or reduced availability of nutrients. Point (3) in Table 2 gives the possibility of slow growing plants, that are difficult to exploit by nitrogen-dependant insects. This can be combined with quantitative defense such as resins. The supply of aromatic amino acids can be minimal. The effect will be greatest in insects with extensive sclerotization. Holometabolic insects have large haemolymph stores in the last larval instar, but hemimetabola need them at every moult. Proteinase inhibitors, present or induced render proteins indigestable.
- Allomones. They have been discussed already. One could add the production of toxic amino acids, such as L-canavanine and γ-aminobutyric acid. The first compound is present in some leguminous seeds. An insect needs specific enzymes to cope with L-canavanine, but it can be expected that this defense can be broken. In fact, an example is the Bruchid beetle *Caryedes brasiliensis*, which has a very active arginase + urease, permitting it to use this amino acid.
- Plants can attrack natural enemies of herbivores (Dicke *et al.*, 1990; Gross, 1981) and in this way substantially contribute to their shield of protection - a cry for help.

Herbivores seem to be able to a great deal of adaptations. But apart from the variety of defense systems, plants can escape in time and space. As mentioned

above, a well-defended reproductive system gives this type of two-fold protection. There is a limited period for the herbivores to attack, and a new generation of the plant species has been dispersed to a potential growing site.

We could also think of a somewhat different terminology: static versus dynamic defense. "Static defense" is for apparent plants, vegetative parts with low tissue value, for plants in harsh conditions (relatively N-independable species). "Dynamic defense" is for the opposite group, and depends on the habitat and changing physiology of the plants. Examples of static defense have been mentioned. Dynamic defense is based on windows of host suitability in time. A few examples may illustrate:

- Plants in favourable habitats (*e.g.*, pioneers) vary strongly in available nitrogen during the season. Generalist insects are more sensitive to variations then those that are taxonomically specialized (McNeill & Prestige, 1982).
- The concentration of defensive compounds can change over the year. Ferns do not make alkaloids or glucosinolates. But ferns can show season-dependent defense. In spring, the cosmopolitan *Pteridium* spp. have a high cyanide content (effective against *Schistocera gregaria*), that is strongly reduced in July. Then, however, defense is taken over by the tannin content and reaches a four-fold concentration.
- Desynchronisation of sensitive growth stages. Apparent plants in stable habitats often use this form of dynamic defense. Winter moths find many oak trees in an unsuitable phase because the buds are still closed. Older leaves are no good food either, and in this way part of the plant is always suboptimal. The frit fly *Oscinella frit*, depends on the stage and nutritive status of grasses and cereals - young larvae need soft plant parts to enter the haulms, but too young parts can not offer suitable food. When different growth stages are mixed, part of the plants can not be infested.

Considering the enormous variety of strategies for defense, it is difficult to assume that insects have a major share in the evolution pressure of plants. Examples have been presented to reconsider a simple two-way effect in these complicated relationships. Even when insects are the overruling stimilus to differentiate defense to answer whatever attack, they first should adapt to novel inventions, and better be quick. Are they always? Perhaps not. A certain biotype of *Aphis fabae* has been reported to prefer *Tropaeolum majus* and feeds on it, but becomes intoxicated and dies (Hille Ris Lambers, 1979). A comparative situation can be found in the role of cucurbitacines in resistance of cucumber plants to spider mites. On many resistant varieties the mites show no sign of deterrence, but obtain a bloated appearance and eventually turn completely black instead of showing the two common darker spots of the intestine. These are filled with black pellets containing indigested food - so they become constipated, and die (De Ponti, 1978).

It can be concluded that co-evolution of plants and insects is intriguing, but by no means fully understood. Even a gross division into apparent and non-apparent plants is questionable. Feeny (1991) discusses the development of qualitative resistance as an active defense to generalists, that can trace less apparent plants in new habitats. He quotes work by Coly, who was unable to detect any difference between apparency of saplings of pioneers and persistents to insects. This induced Coly to develop his "Resource availability" hypothesis. It probably will not be the last terminology invented, and the reader may forgive the use of "static" versus "dynamic" defense in this paper.

A NEW "INVENTIVE" HERBIVORE - *HOMO SAPIENS*

Only a few thousand years ago man arrived at the scene, trying to speed up plant evolution to his advantage. We want plants with a high production of eadible parts, in a an economic agrotechnical system. Domestication of wild plant species, however, usually results in a reduction or loss of natural defensive systems. Energy for defense is directed towards production. As a result we have to decide to either increase the resistance of crops against pathogens and herbivores, or to combat them in some other way.

Herbivorous insects are left with little choice: survive or die and insects that are highly adaptable proved to be able to break our crop protection. It will be clear that there are numerous reasons why particular cultivars of crops are resistant and others susceptible. Resistant cultivars are rarely totally immune from attack; they rather support lower populations of adapted species than do susceptible cultivars. The remaining job can be done by natural enemies. We should realise that Nature does not treat plant defense and natural enemies separately, in reality they interact. Research focussed on either host plant defense or natural enemies, does not take the best of two worlds, as has been discussed above for sap-sucking insects. We often thought to have formed the best possible solution, as Fig. 5 shows. The idea that a bicycle with one big wheel is a perfect invention may bring a compassionate smile on our face - but throwing half of the possibilities of crop protection away by not

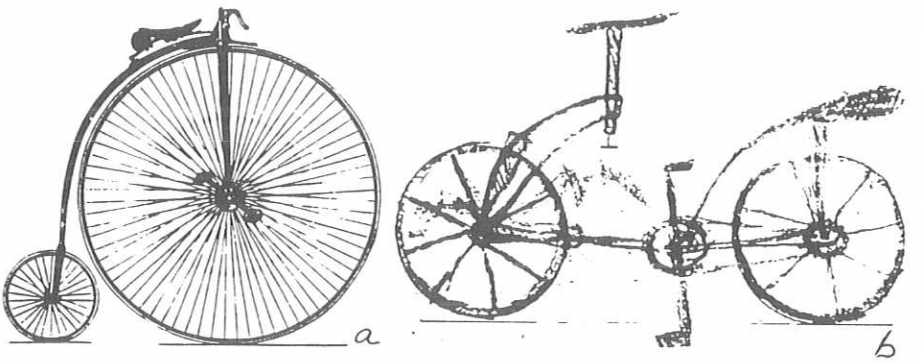

Fig. 5. The "good old ordinary", about 1900 (a) and Leonardo's device, about 1500 (b).

integrating the two major possibilities leaves little to laugh at. Of course, it needs a genius like Leonardo da Vinci who already centuries ago invented a bicycle with two equally sized wheels, as we use them today.

Don't blame the biologists alone. Plants that are reared in monoculture become highly apparent and cannot realize the defenses needed by an apparent and predictable plant (Table 2). Nitrogen fertilization often stimulates the production of alkaloids, but phenolic compounds are usually reduced under lush conditions. Do we want plants that grow under suboptimal circumstances (lower temperature, reduced light)? Very well, but phenolics are best produced under high light regimes.

Attraction of natural enemies can and should be improved; repellent and deterrent plant factors to herbivores should be increased. As long as the herbivores have an alternative (wild) host plant, selection pressure is minimal.

Many novel techniques in science allow us to find answers to these questions - but it often is a type of research that is unapparent and unpredictable - in full contrast to what the politicians responsible for science want us to do.

Poor Tantalus, the son of Zeuss, was once condemned to eternal thurst and hunger, because the water he stood in leveled down and fruits waved away. Once accustomed to a life of luxury, he could not reach what clearly was in front of him.

We can reach an environmentally safe crop protection - it is within our possibilities - and when we don't, it is because of social underdevelopment. In fact, that would be: Tantalus surpassed.

REFERENCES

BALICH, M.J., D.G. FURTH & J. COOPER-DRIVER, 1978 - Biochemical and evolutionary aspects of arthropod predation on ferns. *Oecologia* **35**, 55-89.

BANKS, H.J., 1990 - Physiology and biochemistry of armored scale insects. In: D. Rosen (ed.). *Armored Scale Insects, their Biology, Natural Enemies and Control.* Vol. A, pp. 267-274. Elsevier, Amsterdam.

BARTH, F.G., 1985 - Insects and flowers, the biology of a partnership. Princeton Univ. Press, Princeton, New Jersey.

BERNAYS, E.A., 1978 - Tannins: an alternative viewpoint. *Ent. exp. appl.* **24**, 44-53. (*Proc. 4th Int. Symp. Insect-Plant Relationships*, pp. 244-253).

BERNAYS, E.A., 1982 - The insect on the plant - a closer look. *Proc. 5th Int. Symp. Insect-Plant Relationships*, pp. 3-17. Pudoc, Wageningen.

BERNAYS, E.A., 1991 - Evolution of host-plant specificity. *Proc. 7th Int. Symp. Insect-Plant Relationships*, pp. 313-316. Budapest.

COOPER-DRIVER, G.A.N., 1978 - Insect-fern associations. *Ent. exp. & appl.* 24, 110-116.

DANZIG, E.M., 1980 - Coccoidea of the Far-eastern USSR (Homoptera: Coccineae) with an analysis of the phylogeny of the world fauna of Coccoides. *Nauka Leningradskoe Otdelenie*, 368 pp.

DE PONTI, O.M.B., 1978 - Resistance in *Cucumis sativus* L. to *Tetranychus urticae* Koch. 3. Search for sources of resistance. *Euphytica* 27, 167-176.

DICKE, M., M.W. SABELIS, J. TAHABAYASHI, J. BRUIN & M.A. POSTHUMUS, 1990 - Plant strategies of manipulating predator-prey interactions through allelochemicals: prospects for application in pest control. *Journal of Chemical Ecology* **16**, 3091-3118.

DIXON, A.F.G., 1987 - The way of life of aphids; Host specificity, speciation and distribution. In: A.K. Minks & P. Harrewijn (eds). *Aphids, their Biology, Natural Enemies and Control*, Vol. A, pp. 197-207. Elsevier, Amsterdam.

FEENY, P., 1991 - Theories of plant chemical defense: a brief historical survey. *Proc. 7th Symp. on Plant-Insect Relationships*, pp. 163-175. Budapest.

GOLDSTEIN, W.S., 1987 - Tannin inhibition of cowpea beetle, *Callosobruchus maculatus* F., larval gut ß-glucosidase. *Proc. 6th Int. Symp. on Insect-Plant Relationships*, p. 390, Pau.

GROSS, H.R., 1981 - Employement of kairomones in the management of parasitoids. In: D.A. Nordlund, R.L. Jones & W.J. Lewis (eds). *Semiochemicals, their Role in Pest Control*, pp. 137-150. John Wiley & Sons, New York.

HEIE, O.E., 1987 - Palaeontology and phylogeny of aphids. In: A.K. Minks & P. Harrewijn (eds). *Aphids, their Biology, Natural Enemies and Control*, Vol. , pp. 367-391. Elsevier, Amsterdam.

HILLE RIS LAMBERS, D., 1979 - Aphids as botanists? *Symbolae Botanicaeae Upsaliensis* **22**, 114-119.

JERMY, T., 1991 - Evolutionary interpretations of insect-plant relationships - a closer look. *Proc. 7th Int. Symp. Insect-Plant Relationships*, pp. 301-311. Budapest.

JOHNSON, W.T. & H.H. LYON, 1976 - Insects that feed on trees and shrubs. Comstock Publishing Associates, Cornell University Press, Ithaca, New York, 464 pp.

KATO, M., 1978 - Phenols as indispensible compounds of the synthetic diet of the silkworm *Bombyx mori*. *Ent. exp. & appl.* **24**, 485-490.

LANTON, J.H. & S. McNEILL, 1979 - Between the devil and the deep blue sea: on the problem of being a herbivore. In: *Population Dynamics*, pp. 223-244. 20th Symp. British Ecol. Society, London.

MacKENZIE, A. & A.F.G. DIXON, 1991 - An ecological perspective of host alternation in aphids (Homoptera: Aphidinea: Aphididae). *Entomol. Gener.* **16**, 265-284.

McNEILL, S. & S. PRESTIGE, 1982 - Plant nutritional strategies and insect herbivore community dynamics. *Proc. 5th Int. Symp. Insect-Plant Relationships*, pp. 225-235, Wageningen.

MILES, P.W., 1987 - Plant-sucking bugs can remove the contents of cells without mechanical damage. *Experienta* **43**, 937-939.

REDAK, R.A. & R.G. CATES, 1984 - Douglas-fir *Pseudotsuga menziesii* and spruce budworm *Choristoneura occidentalis* interactions: the effect of nutrition, chemical defense, tissue phenology, and tree physical parameters on budworm success. *Oecologia* **62**, 61-67.

SANDERS, C.J., 1991. Biology of North American spruce budworms. In: L.P.S. van der Geest & H.H. Evenhuis (eds). *Tortricid Pests, their Biology, Natural Enemies and Control*, pp. 579-620. Elsevier, Amsterdam.

SCHLYTER, F. & J. LÖFQVIST, 1990 - Colonization pattern in the pine shoot beetle, *Tomicus piniperda*: effects of host declination, structure and presence of conspecifics. *Ent. exp. appl.* **54**, 163-172.

SCHOONHOVEN, L.M., 1991 - Insects and host plants. 100 years of "botanical instinct". *Proc. 7th Int. Symp. Insect-Plant Relationships*, pp. 3-17. Budapest.

STRONG, D.R., 1979 - Biogeographic dynamics of insect-host plant communities. *Annual Review of Entomology* 24, 89-119.

VAN LOON, J.J.A., 1988 - Sensory and nutritional effects of amino acids and phenolic plant compounds on the caterpillars of two *Pieris* species. Thesis University of Wageningen, pp. 169-204.

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

VELTHUIS, H.H.W., 1991 - Pollen digestion and its relation to the evolution of sociality in the bees. *Proc. Exper. & Appl. Entomol.*, N.E.V., Amsterdam, 2, 2-7.

WINK, M., T. HARTMANN, L. WITTE & J. RHEINHEIMER, 1982 - Interrelationship between quinolizidine alkaloid producing legumes and infesting insects: Exploitation of the alkaloid-containing phloem sap of *Cytisus scoparius* by the broom aphid *Aphis cytisorum*. *Z. Naturforsch.* 37, 1081-1086.

THE IMPACT OF PLANT-DERIVED TERPENOIDS ON BEHAVIOUR AND DEVELOPMENT OF APHIDIDAE

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Key words: Farnesenes, *Matricaria*, mevalonate metabolism, monoterpenes

Summary

Insects synthesize terpenoids with pheromonal and hormonal action. Evidence is now provided that potential host plants also produce terpenoids that affect behaviour and development of Aphididae.

INTRODUCTION

Terpenoids are produced by members of the Plant and Animal Kingdom and are involved in many regulatory systems within or between individuals of the same or different species. They are synthesized via the mevalonate pathway and terpenoids produced in nature have functions ranging from internal messengers to defensive compounds, both repellants and toxins, and semiochemicals.

Terpenoids are derived from mevalonic acid, the synthesis of which is dependent on a key enzyme, hydroxymethylglutaryl co-enzyme A reductase. Substances that inhibit or activate this key enzyme can have a profound effect upon the production of the terpenoids. Goldstein & Brown (1990) have elucidated a great deal of the regulatory system of mevalonate metabolism in mammals. Isoprenoid end products such as steroids and other mevalonate metabolites operate in a multivalent feed-back system to suppress the activity of HMG CoA reductase. This knowledge is particularly important with regard to the biosynthesis of isoprenoids that are essential for cell growth and division, and steroid hormones.

In insects terpenoids may act as endocrine hormones (the juvenile hormones), as pheromones, as compounds affecting feeding behaviour and as semiochemicals (Gut *et al.*, 1987). Plant-derived terpenoids can affect behaviour and development of phytophagous insects: *e.g.*, sesquiterpene lactones act as repellents to storage beetles, can paralyse locusts and (E,E)-farnesol (a sesquiterpene alcohol) has a hormonal effect on many insect species (Van Oosten *et al.*, 1990).

Aphididae have a specific relation with their host plants. Most aphid species feed on phloem sap of a limited number of hosts. In this way they avoid defensive compounds (allomones) that render the plant uneatable by insects that use the contents of epidermal or mesophyllic cells. The limited number of allomones that can be transported in the phloem (such as phenolics) do not deter all aphid species from feeding, as some species can detoxify these allomones and may even use them as kairomones. Terpenoids, however, are mainly involved at a different site of the

defensive system of plants to insects. The wild potato species *Solanum berthaultii* is resistant to aphids and the leaves have glandular hairs releasing a high amount of terpenoids, including (E)-ß-farnesene, which is the alarm pheromone of aphids. Aphids produce both monoterpenes (that can act as sex pheromones) and sesquiterpenes, some of which have a function as semiochemicals or hormones.

Terpenes are involved in morphogenesis of aphids. High levels of JH_{III} probably induce the production of virginoparae (Hardie, 1987). The ratio and levels of farnesene isomers show marked differences that are specific for particular morphs, whereas exogenous application of farnesenes affect the course of development (Harrewijn *et al.*, 1991; Gut *et al.*, 1991). Different plant parts are characterized by specific farnesene isomers, but also by inhibitors of these compounds masking their potential effect on phloem feeding insects that do not disrupt compartmentation.

These facts rise the question how terpenoids derived from non-host plants could affect behaviour and development of aphids.

MATERIALS AND METHODS

- **Aphids**

Stock cultures of *Myzus persicae* (Sulz.) were maintained on Chinese cabbage, *Aphis fabae* (Scop.), *Acyrthosiphon pisum* (Harris) and *Megoura viciae* (Buckton) were on broad bean plants in a climatized glasshouse at 19 ± 2°C, with 16 h photoperiod.

- **Plants and synthetic compounds**

The following plant species were grown outdoors: *Matricaria chamomilla*, *M. perforata*, *M. parthenium* and *M. matricaroides*.

Terpenoids were extracted with highest purity hexane: 10 ml of hexane was ground in a Waring blender with 1.0 g of either fresh or dried (45°C, 24 h) plant material. Extracts were purified on a chromatographic column with florisil of 60-100 mesh (BDH), in some cases after centrifugation at 6000 rpm. The monoterpenes menthone and menthol were obtained from Chemica, cineole from Sigma. Camomile-oil type Deutsch Naturell was provided by Roth (Germany).

- **Analyses**

Sesquiterpenes were estimated with a Varian 3700 GC, equipped with a wide-bore CP Sil 5 CB column of 0.5 mm id., 25 m long and a He-flow of 7 ml/min, T 140°C, isothermic. Peaks were compared with those of portions of synthetic farnesene isomers.

- **Aphid treatments**

The effect of vapour on plant-aphid combinations was estimated by blowing air through a Pasteur pipette containing a calculated amount of plant extract identical with 50 and 500 ng (E)-ß-farnesene. Application of monoterpenes by spraying was done with a Desaga vaporisator filled with 0.5 and 1% solutions in acetone. The aphids were placed on a filter paper disc (ø 11 cm) on a turntable (16 rpm) and received 100-200 ng at a body weight of 300 µg. Application of sesquiterpenes was done by covering the body of aphids with 0.01 µl of 1% solutions in isopropylalcohol or acetone, resulting in 100 ng per aphid of 300 µg. To that end the aphids were placed on a vacuum-operated microsurgery table, equipped with a Fonbrune micro-injection device. Camomile-oil was applied in the same way, using 0.01 µl of a 1% solution in acetone, resulting in 15 ng (E)-ß-farnesene in 100 ng of oil. After

treatments the aphids were transferred to their natural host plants.

RESULTS

Table 1. Terpenoid content of Matricaria *species and response of* M. persicae *(M_3)*

	M. chamomilla	*M. matricaroides*	*M. parthenium*	*M. perforata*
Flowers				
(E)-ß-farnesene	++	+++	+	++
(Z,E)-α-farnesene	-	-	-	+
add. terpenoids	+	+	+++	+
alarm response	-	++	-	+
Leaves and stems				
(E)-ß-farnesene	+++	++	+	++
(Z,E)-α-farnesene	+/-	-	+	+
add. terpenoids	+	+++	+++	++
alarm response	-	++	-	-
Roots				
(E)-ß-farnesene	++	+	+	++
(Z,E)-α-farnesene	-	+	+	+
add. terpenoids	+	+	+	++
alarm response	+	++	-	+

Symbols terpenoids: - not present (detectable); + 0-25 μg/g fresh material; ++ 25-50 μg; +++ > 50 μg; alarm response: - no response; + moderate response (≤ 50%); ++ strong response (≥ 50%).

Table 1 shows that the four *Matricaria* species produce different amounts and ratios of farnesenes and that the distribution over plant parts is species-dependent. The flowers and the roots of *M. perforata* contain a considerable amount of (Z,E)-α-farnesene. The content of (E)-ß-farnesene of all parts of *M. parthenium* is low compared with *M. chamomilla* and relatively high in the two other species, except for the roots. Aphids do not respond to vapour of extracts of *M. parthenium*; these plants are characterized by a high amount of terpenoid compounds, that inhibit the alarm reaction. *A. pisum* reacted with an alarm response on almost all extracts in which (E)-ß-farnesene was present, including the inpurified camomile-oil. The biotypes M_2 and M_3 of *M. persicae* did not respond to the oil, indicating that it contains inhibitors of the alarm pheromone. Purification on a florisil column resulted in an immediate reaction of *M. persicae*. This means that the inhibitors are not identical with humulene or caryophyllene, known to suppress the alarm response (Dawson *et al.*, 1984), as these compounds eluate from a florisil column. The GC spectra and the blue colour of the fractions did not reveal the presence of any of the two but pointed towards chamazulene. Generally speaking, flowers and roots show little activity of inhibitors, but leaf material does. Indeed chamazulene is absent from the roots of camomile plants (Reichling *et al.*, 1979). It should be noted that *M. matricaroides* contains mainly (E)-ß-farnesene and possibly germacrene-D, that did

not interfere with the alarm response. A closely related substance, germacrene-A is known for its alarm effect upon some aphid species (Bowers *et al.*, 1977). The extracts of *M. parthenium* contains many mono- and sesquiterpenes other than farnesenes.

Table 2. Percentage mortality of adult M. persicae *(biotype M_3) after application of camomile-oil, dissolved in acetone*

	after 2 h		after 6 h	
	paralysed	dead	paralysed	dead
Control (acetone)	0	0	0	0
camomile-oil 1%	5	33	0	100

Table 2 shows that 100 ng of camomile-oil contains sufficient substances that excert a lethal effect upon *M. persicae*. As not more than 15 ng of (E)-ß-farnesene was applied, the effect is completely different from thanatosis due to farnesenes, as described by Van Oosten *et al.* (1990). Apparently some terpenoids are toxic upon contact with the aphids. Only a small proportion gets paralysed and not a single individual recovers. *M. persicae* and *M. viciae* sprayed with menthol showed a disturbed feeding behaviour for about 24 h. They refused to feed for most of the time, but larviposition was about twice that of the non-treated or acetone-treated aphids. Normally, non-feeding aphids hardly produce offspring. *M. viciae* switched to cannibalism: they tried to feed upon each other. Although specialized morphs of aphids can attack natural enemies, cannibalism is not described and must be the result of the menthol treatment. Higher doses of this cyclic monoterpene are lethal. The effect of menthone is much less pronounced, it does not induce cannibalism, but has a lethal effect above 4%. Half of the larvae of both the menthol- and menthone-treated aphids die in their second or third larval stage.

Fig. 1 shows the course of development of batches of larvae, collected every two days. There is a striking reduction in the number of alatae among the first batch of larvae produced by aphids treated with farnesenes. As the larvae were crowded 25 per clipcage of 20 mm ø usually about 50% takes the alate course of development, and this proportion increases in the subsequent batches. Obviously the morph-active effect of farnesenes is temporarily and expresses itself for about 48 h. In the subsequent batches even more alatae are present, but this can be explained by their high numbers. In addition to the apterizing effect of the terpenoids, they strongly stimulate larviposition and this effect lasts throughout all batches. The average daily offspring of the control aphids was 1.5, of the (E)-ß-farnesene treated ones 2.8 (stat. diff. with $P<0.01$), of (Z,E)-α-farnesene 2.6 (not diff. from 2.8), and of the (E)-ß-farnesene + cineole 3.7 (diff. with $P<0.01$ from all others). Especially the high reproduction rate induced by cineole addition was characterized by smaller larvae. In all cases mortality among the offspring was less than 2%.

DISCUSSION

Aphids have a highly active and differentiated mevalonate metabolism (Gut *et*

al., 1991) and it is likely that in these insects feed-back systems exist for the regulation of alle these different branches. In non-sterologenic cells as present in insects, the regulatory mechanism is not necessarily the same as in mammals. Havel *et al.* (1986), in their work with *Drosophila* had strong evidence that biosynthesis of mevalonic acid can take more than one pathway (mevalonate shunt metabolism). Nevertheless, non-sterologenic cell systems are also regulated by isoprenoid end products. An example is the suppression of endogenic JH_{III} synthesis by exogenic application of JH_{III}, which is an isoprenoid end product (Feyereisen, 1984).

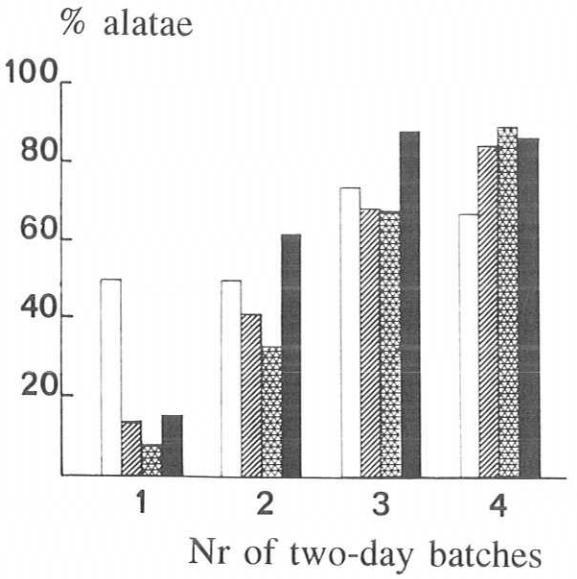

Fig. 1. Production of winged morphs by M. persicae *treated with terpenoids. Control* □ *; (E)-ß-farnesene* ▨*; (Z,E)-α-farnesene* ▩*; (E)-ß-farnesene + cineole* ■ .

Farnesenes, like JH_{III}, could act directly on the endogenous regulating systems of the mevalonate metabolism. When provided continuously as in artificial diets, they may inhibit HMG-CoA reductase or act at the level of farnesyl pyrophosphate causing reduced JH_{III} titre and sexual production (Gut *et al.*, 1991). A single application of pure farnesenes, however, is only effective for a limited period (48 h) during which the effect is identical with continued treatment of adult *A. fabae*: inhibition of the production of winged offspring (Van Oosten *et al.*, 1990). This is not caused by retention of winged morphs at birth, as the reproductive rate is stimulated and not reduced.

Various ant species that exploit aphids apply (Z,E)-α-farnesene from mandibular or Dufours glands to individuals that subsequently produce apterae (Ali, 1987). These facts suggest that plant-derived terpenoids may act on the central nerve system and terpenoids are proved reversible inhibitors of acetylcholinesterase and can cause paralysis and mortality in nonadapted insects (Ryan & Byrne, 1988). Insects that eat plants are successful in detoxifying cholinergic inhibitors, but aphids are not used to acquisition of terpenoids. This may explain their sensitivity to plant farnesenes, that

have an effect on behaviour when pure, in low dose, and not contaminated by compounds such as humulene and caryophyllene that are known to inhibit their action. In camomile-oil terpene alcohols can act as synergists, rendering it toxic to aphids. Monoterpenes like menthol appear to affect both behaviour and survival, making them interesting in the study of host-plant resistance or as aphicides not toxic to higher animals. The fascinating effect of cineole (either alone or in combination with farnesenes) remains to be further investigated.

LITERATURE

ALI, F.M., A.B. ATTYGALE, E.D. MORGAN & J.P.J. BILLEN, 1987 - The Dufour gland substances of the workers of *Formica fusca* and *Formica lemani* (Hymenoptera: Formicidae). *Comp. Biochem. Physiol.* **88B**, 59-63.

BOWERS, W.S., C. NISHINO, M.E. MONTGOMERY, L.R. NAULT & M.W. NIELSON, 1977 - Sesquiterpene progenitor, germacrene A: an alarm pheromone in aphids. *Science* **196**, 680-681.

DAWSON, G.W., D.C. GRIFFITHS, J.A. PICKETT, M.C. SMITH & C.M. WOODCOCK, 1984 - Natural inhibition of the aphid alarm pheromone. *Entomol. exp. appl.* **36**, 197-199.

FEYEREISEN, R., R.P. RUEGG & S.S. TOBE, 1984 - Juvenile hormone III biosynthesis. Stoichiometric incorporation of [2-^{14}C] acetate and effects of exogenous farnesol and farnesoic acid. *Insect Biochem.* 14, 657-661.

GOLDSTEIN, J.L. & M.S. BROWN, 1990 - Regulation of the mevalonate pathway. *Nature* **343**, 425-430.

GUT, J., P. HARREWIJN, A.M. VAN OOSTEN & B. VAN RHEENEN, 1987 - Additional function of alarm pheromones in development processes of aphids. *Meded. Fac. Landbouww. Gent* **52**, 371-378.

GUT, J., P. HARREWIJN, A.M. VAN OOSTEN & P.G.M. PIRON, 1991 - Terpenes as regulating factors in aphid morphology. *Proc. Conf. Insect Chem. Ecol., Tábor, 1990.* pp. 347-360. Acad. Prague Publ., The Hague.

HARDIE, J., 1987 - Neurosecretory and endocrine systems. In: A.K. Minks & P. Harrewijn (eds). *Aphids, their Biology, Natural Enemies and Control*, pp. 139-152. Elsevier, Amsterdam.

HARREWIJN, P., P.G.M. PIRON, J. GUT & A.M. VAN OOSTEN, 1991 - The role of terpenoid endproducts in development of the aphid *Megoura viciae* (Buckton). *Proc. Exp. & Appl. Ned. Entomol. Ver. Amsterdam* **2**, 74-79.

HAVEL, C.M., E.R. RECTOR II & J.A. WATSON, 1986 - Isopentenoid synthesis in isolated embryonic *Drosophila* cells. Possible regulation of 3-hydroxy-3-methyl glutaryl coenzyme A reductase activity by shunted mevalonate carbon. *J. Biol. Chem.* **262**, 10150-10156.

VAN OOSTEN, A.M., J. GUT, P. HARREWIJN & P.G.M. PIRON, 1990 - Role of farnesene isomers and other terpenoids in the development of different morphs and forms of the aphids *Aphis fabae* and *Myzus persicae*. *Acta Phytopath. et Entomol. Hung.* **25**, 331-342.

REICHLING, J., R. BEIDERBECK & H. BECKER, 1979 - Vergleichende Untersuchungen über sekundäre Inhaltsstoffe bei Pflanzentumoren, Blüten, Kraut und Wurzeln von *Matricaria chamomilla* L. *Planta Medica* **36**, 322-332.

CHRYSANTHEMUM RESISTANCE TO TWO TYPES OF THRIPS (*Frankliniella occidentalis Pergande*) FEEDING DAMAGE

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Key words: host-plant resistance, *Frankliniella occidentalis*, *Chrysanthemum*

Summary

The resistance of 43 chrysanthemum cultivars to western flower thrips *Frankliniella occidentalis* was investigated in the laboratory. Two types of thrips feeding damage (silver damage and growing damage) were measured for four weeks. Significant differences in growing damage and silver damage were found from the first week on. The cultivar ranking of all weeks were strongly correlated. The frequency of both the silver and growing damage was Log normal distributed. No correlation was found between the amount of silver damage and growing damage. This suggests that there are different resistance mechanisms involved. A promising group of cultivars with low levels of both silver damage and growing damage was found.

INTRODUCTION

Pesticides, used for crop protection are a major threat to the environment. The use of pest resistant crops may reduce the use of chemicals by slowing down the population growth of insects (De Ponti, 1982).

Chrysanthemum (*Dendranthema grandiflora* Tzvelev.) is an important ornamental crop in the dutch economy but its production is threatened by the thrips species *Frankliniella occidentalis* (Pergande) which is a major pest on this and other crops. A project has been initiated to develop a biotechnological test to scan chrysanthemum seedlings for resistance to pests such as *F. occidentalis* and *Liriomyza trifolii* (Burgess). In order to develop a resistance test the mechanisms of resistance need to be studied in detail.

A method was developed to assess thrips resistance in chrysanthemums (De Jager et al, 1993). This study also showed that differences in thrips resistance were present in the five cultivars studied. To study the mechanisms of resistance a selection of cultivars have to be made. Therefore the variation in thrips resistance of 43 different chrysanthemum cultivars is studied in a choice experiment. As a measure of resistance the amount of feeding damage is taken.

When the thrips feeds on developing tissues, affected cells are unable to expand, so leaves and petals become distorted upon subsequent growth. We will refer to this type of damage as "growing" damage. Feeding on expanded tissues causes cells to become filled with air, which imparts a silvery appearance to the affected tissue. This type of damage will be referred to as "silver" damage.

An other important question is if there is any correlation between silver damage and growing damage. When the same character determines the resistance of the young (unexpanded) and old leaves, a positive correlation between silver and growing damage would be found. In that case it would be sufficient to measure one type of damage, which

is less time consuming. However, a negative correlation is also possible in case of preference of older leaves or younger leaves. Then, damage of the unpreferred leaves occur when the preferred leaves are of a bad quality. This would give a lot of difficulties for breeding thrips resistant chrysanthemums.

MATERIAL AND METHODS

Fortythree different chrysanthemum cultivars which were thought to vary in thrips resistance were selected. From each cultivar, 12 commercially produced cuttings were grown for four weeks in a growth room which was programmed for 20°C,70% RH and a 16L:8D photoperiod.

After four weeks per plant 7 adult female thrips were released in the growth room. The photoperiod of the growth room was changed to 8L:16D at the beginning of the experiment. For four weeks, every week the thrips feeding damage was estimated. The silver damage was assessed by counting the number of feeding scars of 1,5 mm^2 on one third of the leaves. For scar counting leaf number two and subsequently every third leaf, till the unfolded top leaves were taken. The growing damage was assessed by counting the number of leaves with this type of damage. The data were analysed by ANOVA.

The thrips came from a strain that has been reared for several months on the flowers of the chrysanthemum cultivar FR 16 (cv 8). The thrips rearing conditions were 20°C day and night temperature, 70% RH and a 12L:12D photoperiod.

RESULTS

Significant differences in growing damage and silver damage were found from the first week on. The significance increased during the experiment for both silver damage (week 1: $F= 12.603$, $p<0.0001$; week 4: $F=30.758$, $p<0.0001$) and growing damage (week 1: $F=5.695$, $p<0.0001$; week 4: $F=93.995$, $p<0.0001$). The cultivar ranking of all weeks were strongly correlated.

After four weeks the silver damage on the most susceptible cultivar was 76 times higher as on the most resistant cultivar (fig 1a). The growing damage varied between 0 and 11 leaves per plant (fig. 1b).

The frequency of both the silver and growing damage was Log normal distributed.

No correlation was found between the growing damage and the silver damage (fig. 2b). A group of cultivars with resistance to both silver damage and growing damage was found (fig. 2a).

DISCUSSION

The differences in thrips damage between the cultivars are very clear and therefore it is possible to select some cultivars to study the modality of the thrips resistance (antixenosis, antibiosis, tolerance). The selected cultivars will be investigated in choice and non choice experiments in which the differences in feeding damage and thrips population growth are determined.

No correlation was found between the amount of silver damage and the amount of growing damage. This suggests that there are different resistance mechanisms involved. The absence or presence of silver or growing damage is determined by the preference of the thrips for the expanded or the unexpanded (top) leaves.

A promising group of cultivars with low levels of both silver damage and growing damage is found. For the breeding companies this means that it is possible to select for both silver damage and growing damage resistance.

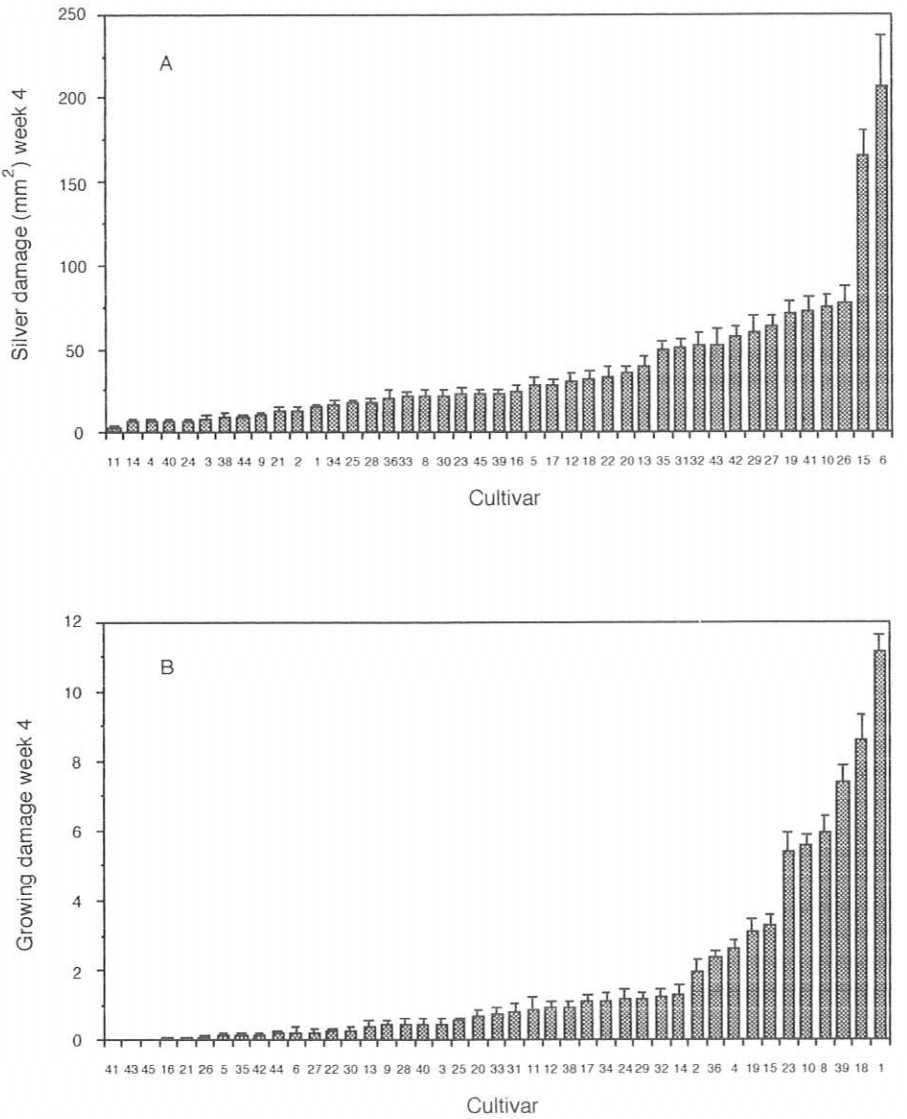

Figure 1. Silver damage (A) and growing damage (B) caused by F. occidentalis after four weeks on 43 different chrysanthemum cultivars

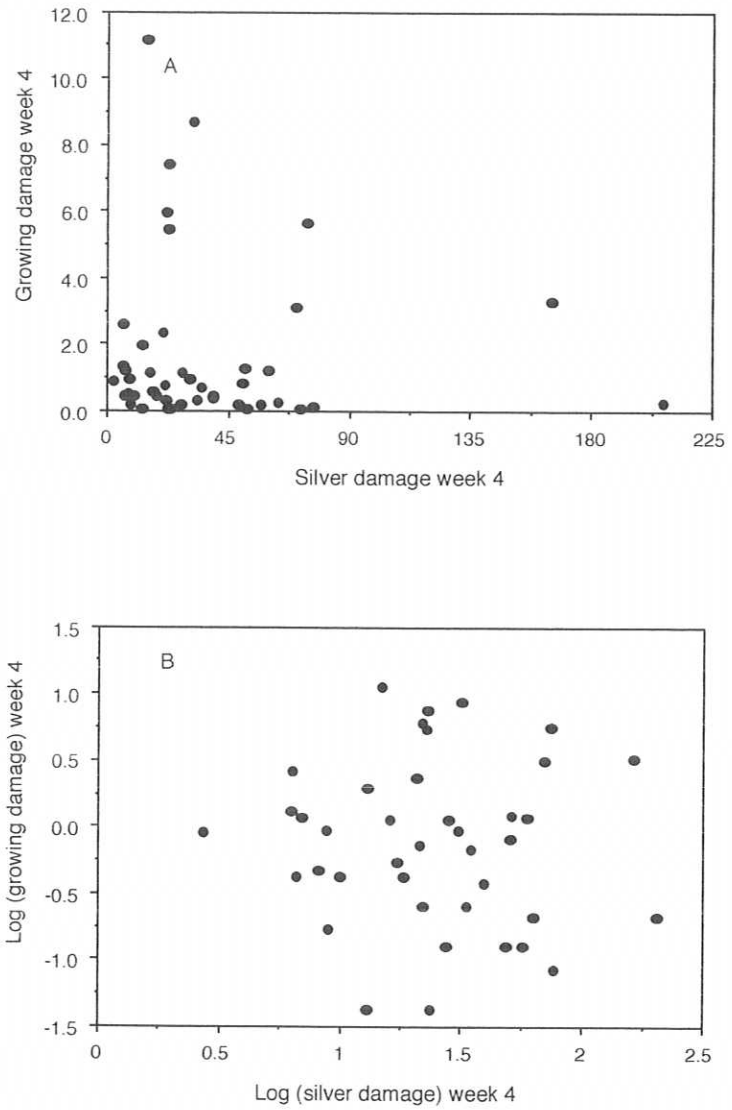

Figure 2. Correlation between silver damage and growing damage caused by F. occidentalis on 43 different chrysanthemum cultivars (A). After log transformation (B).

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REFERENCES

DE JAGER, C.M., R.P.T. BUTÔT, T.J. DE JONG, P.G.L. KLINKHAMER & E. VAN DER MEIJDEN, 1993 - Population growth and survival of western flower thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on different chrysanthemum cultivars: two methods for measuring resistance. *J. Appl. Ent*, in press.

DE PONTI, O.M.B., 1982 - Plant resistance to insects: a challenge to plant breeders and entomologists. in: "*Proc. 5th int. Symp. Insect-Plant Relationships*", Eds. J.H. Visser and A.K. Minks, Pudoc, Wageningen, 337-347.

COMPARATIVE APPROACH TO INFOCHEMICAL USE BY PARASITOIDS FOR THE CASE OF *COTESIA GLOMERATA* AND *COTESIA RUBECULA*

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Key words: parasitoids, searching behaviour, infochemical use, generalist, specialist

Summary

Parasitic wasps, that search for their hosts, may use chemical information derived from plants, the first trophic level, and the herbivorous hosts, the second trophic level. These chemical stimuli may contain general and specific components. To test our hypothesis, that the host-specificity of a parasitoid species determines the searching strategy, a comparative research was initiated on the tritrophic system cabbage (*Brassicaceae*), cabbage-white caterpillars (*Pieris* sp.) and two closely related parasitoid species of these herbivores, the generalist *Cotesia glomerata* and the specialist *C. rubecula*. A theoretical outline will be given here, illustrated with preliminary results.

BACKGROUND

To understand the interactions between natural enemies and their herbivorous hosts and between herbivores and host plants, theory and research on these interactions should be considered within a tritrophic context (Price et al., 1980). As shown by research in the last 15 years (Vinson, 1976; Turlings et al., 1990; Dicke et al., 1990; Vet & Dicke, 1992), tritrophy is an important concept for studying the searching process of natural enemies. Parasitoids and predators have evolved various host-searching strategies (Price, 1981; van Alphen & Vet, 1986; Lewis et al., 1990), resulting in a high diversity of specialized lifestyles. Nevertheless, general aspects in foraging behaviour have been found. While searching, natural enemies respond to various stimuli emanating from different trophic levels in their environment. In the process of host location, chemical information often plays an important role; plant derived chemicals being important in attracting natural enemies at longer distances (Vinson, 1981) and host derived stimuli mediating directed response when the distance to the host gets smaller (Weseloh, 1981).

An important aspect of parasitoid attraction by a herbivore-attacked plant via chemical stimuli, is the efficiency of the foraging strategy of insect parasitoids. A high variability in searching behaviour between parasitoid species has been observed (van Alphen & Vet, 1986), and the question rises what the adaptive value of differences in foraging strategies of parasitoids may be. A recent hypothesis states, that part of this variability in foraging behaviour is affected by the degree of specialization of parasitoids (Vet & Dicke, 1992). This hypothesis will be tested in a tritrophic system consisting of cabbage (*Brassicaceae*) - cabbage-white caterpillars (*Pieris* sp.) and two closely related larval endoparasitoids of these caterpillars (*Cotesia glomerata* and *C. rubecula*).

THE PRESENT QUESTION

The evolution of foraging strategies of insect parasitoids is constrained by the ability of females to find the second trophic level, the hosts (Vet & Dicke, 1992). Insect

parasitoids that search for hosts, can both use chemical information derived from their hosts and the host's foodplant. The usability of this information relates to its reliability and detectability, where reliable stimuli ideally give information on the presence, accessibility and suitability of hosts (Vet et al., 1991) and where detectability of stimuli is a function of the dimension of the stimulus source, distance and medium between source and receptor, and the sensitivity of the receptor (Wäckers & Lewis, 1993). Information from hosts is the most reliable information, but at longer distances hardly detectable (Turlings et al., 1991). This is due to the relatively small biomass that hosts represent in their environment, and by the continuous selection on inconspicuousness of herbivores to their natural enemies (Vet et al., 1991). On the other hand, chemical information from the host's foodplant will have a higher detectability, because of the larger biomass, but its reliability is much less, compared to host-derived stimuli. Whether stimuli from plants are reliable, depends on the probability of infestation of the plant, and the degree of infestation (Vet et al., 1991). A main aspect of efficiency of foraging strategies will be the use of information from the plant-host complex that is both reliable and detectable (Vet & Dicke, 1992). Parasitoids have evolved different strategies to solve this reliability-detectability problem, of which two will be of main interest in the present study. First there is the use of herbivore-induced synomones by parasitoids; specific volatiles, that are released by the plant upon herbivore attack, as a means of indirect defense. Secondly, associative learning, where highly detectable stimuli are associated with highly reliable stimuli, may be an important aspect of the parasitoid's foraging strategy. A third solution may be the infochemical detour, where the parasitoid resorts to the use of more detectable stimuli derived from host stages other than the one under attack (Vet et al., 1991), an aspect of less importance for the present study.

hypothesis

The information needed during foraging by parasitoids, is expected to depend on the rate of specialization on the first and second trophic level of the parasitoid species. For parasitoids that attack a wide variety of hosts on the same plant species, information on the identity of hosts is relatively unimportant, compared to the more specialized species. In the case of specialization on the first trophic level, the plant, specific information of the plant becomes more important when hosts occur only on one plant species, than when hosts live on different plant species (Vet & Dicke, 1992).

We hypothesize, that the behaviour of specialists on the 1st ánd 2nd trophic level is more congenitally fixed, and better adapted to specific stimuli from the plant-host complex as a result of the close association with its host. For generalists a fixed behaviour does not seem to be functional and therefore not likely to exist. Associative learning is expected to be a more important factor in host searching behaviour of generalist species, especially when they are generalists at the plant level (Vet & Dicke, 1992).The adaptive value of differences in foraging strategies can only be determined through comparisons between closely related species (Vet & van Alphen, 1985). The study discussed in this paper intends to test the hypothesis through a comparison of foraging strategies of two closely related parasitoid species from the cabbage - *Pieris* - *Cotesia* complex. *Cotesia glomerata* is a gregarious and polyphagous species, attacking larvae of the large and small cabbage white (*Pieris brassicae* and *Pieris rapae*) and the small veined white (*P. napi*), mainly feeding on cruciferous host plants, but also the large veined white (*Aporia crataegi*), that occurs on *Rosaceae*. The related *C. rubecula*

is a solitary and specialist parasitoid of *P. rapae* that feeds on cruciferous plants (Laing & Levin, 1982).

aims and preliminary results

The aims of this study are to determine 1) the specificity of the information used by the generalist and the specialist parasitoid species, 2) the variability in behavioural responses of both species due to learning, and 3) a correlation between the foraging decisions of parasitoid females and the suitability of a potential host on different host plants and consequently for their fitness. In the following the aims will be discussed in more detail and illustrated with preliminary results.

ad 1. When parasitoids are able to respond to specific stimuli, caused by the feeding behaviour of their 'preferred' hosts on a suitable foodplant, and thus to discriminate between profitable and non-profitable host-habitats, we expect this to enhance their foraging effectiveness. *Cotesia glomerata* is expected to use more general stimuli, while for *C. rubecula* use of specific *P. rapae*-cabbage-related stimuli would be functional. To elucidate the specificity of the stimuli used, differences in response to various odour sources will be determined, as well as odour preferences for certain plant-host combinations of both parasitoid species.

ad 2. Learning is a common phenomenon in insect parasitoids (Vet & Groenewold, 1990). According to our hypothesis, the rate at which stimuli can be learned and will be learned may depend on the specialization rate of the parasitoids. Differences in the degree to which behaviour is innate or can be altered through experience are important to explain variability in behaviour between parasitoid species. The specialist *Cotesia rubecula* is expected to have a more fixed behaviour towards 'preferred' specific stimuli, while *C. glomerata* is expected to show a more flexible behaviour, mediated through odour learning.

In a semi-field set-up (Steinberg et al., 1992) dual-choice situations were used to establish the influence of pre-flight experience on the female's subsequent choice. When individual *Cotesia glomerata* were given a contact experience on a host-damaged leaf (without larvae, but with host by-products) of a certain plant-host complex, we see that these females have a significant preference for the complex they had experienced when offered against another, 'new', plant-host complex (fig. 1). In this species preference can be altered through an experience with host-damaged leaf material. Similar results were obtained when the reward consisted of oviposition.

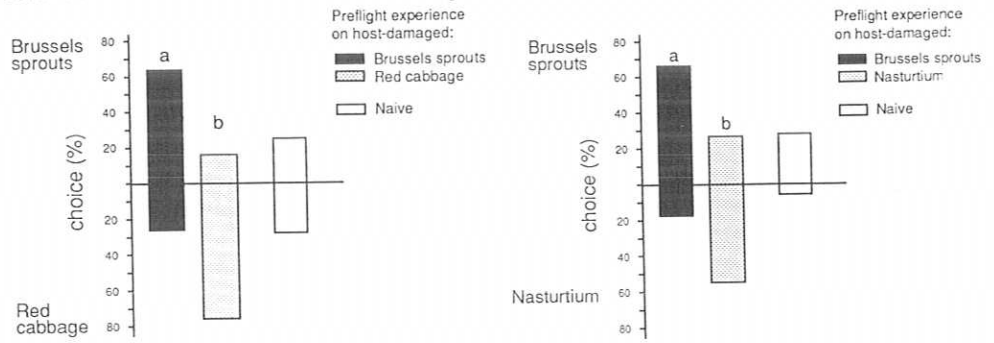

Figure 1. Influence of different preflight experiences on the choice for certain plant-host complexes for Cotesia glomerata. Different letters indicate P<0,05.

In contrast, *Cotesia rubecula* females with a contact experience on a cabbage leaf damaged by *P. rapae* larvae, did not show such a switch in preference. Instead they chose significantly for brussels sprouts (fig. 2a). When females were rewarded with 3 subsequent ovipositions in *P. rapae* larvae on a red cabbage plant, still no preference for that plant-host complex was observed (fig. 2b). Apparently, an innate preference and a fixed response exist toward the *P. rapae* - brussels sprouts complex in this parasitoid species.

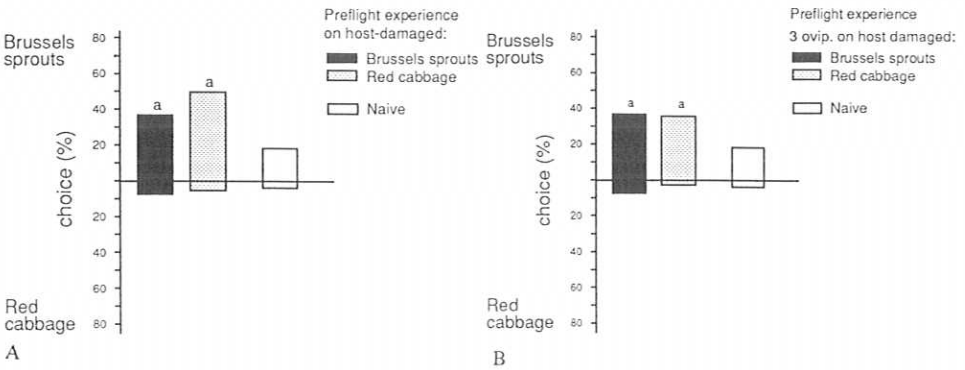

Figure 2. Influence of different preflight experiences on the choice for certain plant-host complexes for Cotesia rubecula. Different letters indicate P<0,05.

These preliminary results give an indication on the correlation between a parasitoid's host specificity and her foraging decisions, since in the specialist *Cotesia rubecula* no odour learning was observed, while this was evident in the generalist parasitoid *C. glomerata.*

ad 3. A better insight in the foraging strategies of parasitoids should include the suitability of the hosts for the development of the parasitoid larvae. Whether and how female parasitoids are able to find the most suitable hosts, the step between host-habitat location and host acceptance, is part of point 1 and 2 in the paragraphs described earlier. Here we want to answer the question whether the female's oviposition preferences are reflected in the expected fitness of her offspring.

Figure 3 shows the acceptance ratios (i.e. the number of ovipositional attempts divided by the number of antennal contacts) of both parasitoid species for three *Pieris* species. *Cotesia glomerata* preferred the younger stages of the three host species over the older stages, with a stronger overall preference for *P. brassicae* larvae. *Cotesia rubecula* showed a different pattern. Concerning *P. brassicae* larvae, females of the specialist parasitoid species had a strong preference for the early first instars, while the third instar larvae are almost completely ignored. Acceptance ratios for *P. napi* larvae were relatively low throughout all developmental stages, but *C. rubecula* showed a clear preference for *P. rapae* larvae, and readily accepted and oviposited in all 6 developmental stages of this species. The latter result may be an attribute of a specialized parasitoid: to extend the range of host age for oviposition, when the number of host species in the diet is limited (Brodeur & Geervliet, 1992).

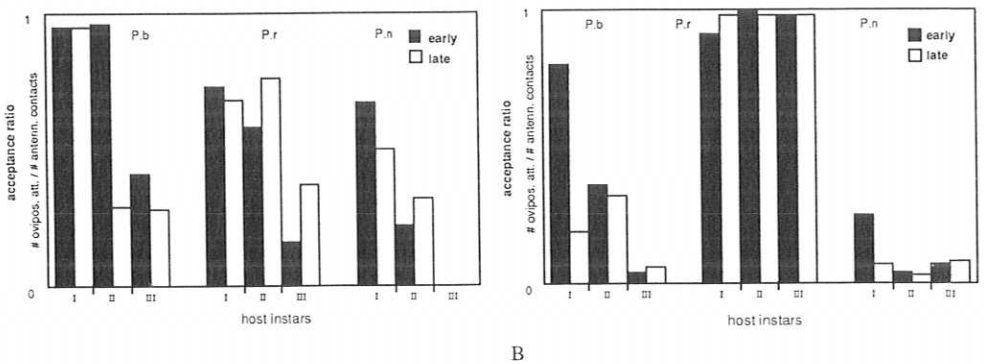

Figure 3. Acceptance ratios for different host species. A: Cotesia glomerata, B: Cotesia rubecula. P.b.=Pieris brassicae, P.r.=Pieris rapae, P.n.=Pieris napi. N=30 females per treatment, except for 3a: P.n late I, and II N=15 females.

At the point of host suitability, besides other parameters adult parasitoid weight has been shown to be significantly correlated with different fitness components for both *Cotesia glomerata* and *C. rubecula* (Nealis et al., 1984; Karowe & Schoonhoven, 1992). Figure 4 shows adult dry weight of the offspring of both parasitoid species in the three host species. For *Cotesia glomerata* no differences in adult weight could be observed, while *C. rubecula* larvae developing in *P. rapae* produced heavier adults, males and females, than those developing in *P. brassicae*. From *P. napi* no offspring of *C. rubecula* could be obtained at all.

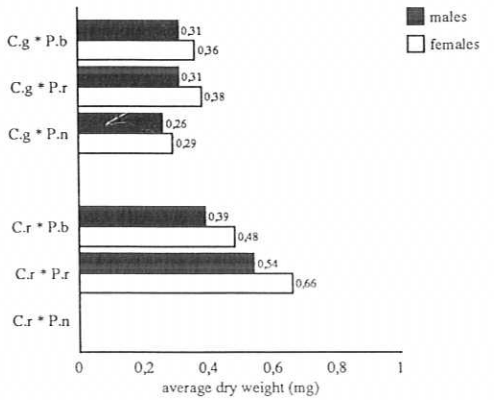

Figure 4. Adult dry weight (mg.) of the offspring of Cotesia glomerata (C.g.) and C. rubecula (C.r.) from different host species. P.b.=Pieris brassicae, P.r.=Pieris rapae, P.n.=Pieris napi. N=30 females per combination for C. glomerata, N=120 for C. rubecula.

With regard to overall suitability, all three host species turned out to be suitable for the generalist *Cotesia glomerata*, while *Pieris rapae* was obviously most suitable to *C. rubecula* (Geervliet & Brodeur, 1992). There seems to be a good correlation between oviposition preferences and host suitability for both *Cotesia* species parasitizing these three host species, as the same host ranks were obtained in both host preference and suitability studies. For these studies the interactions between the 2nd and 3rd trophic level were dealt with, and we are aware that still the 1st level has to be taken into consideration, since it has been shown, that the host plant not only affects the performance of the herbivore on it's food plant, but also the performance of the parasitoid on the herbivore (Karowe & Schoonhoven, 1992).

This comparative approach to foraging strategies of a generalist and specialist parasitoid species is not just interesting from the point of view of fundamental science. This approach is also valuable to biological control. Specificity of natural enemies is often used as a criterium for the selection and application in biocontrol programmes (van Lenteren, 1986). Knowledge about differences in foraging strategies between generalists and specialists may have an additive value to justification of selection criteria in the development of such control programmes.

REFERENCES

ALPHEN, J.J.M. VAN, & L.E.M. VET, 1986 - In: J.K. Waage & D.J. Greathead (Eds.). *Insect parasitoids* 23-61. London, Academic.

BRODEUR, J. & J.B.F. GEERVLIET, 1992 - *Med. Fac. Landbouww. Univ. Gent* **57/2b**, 543-546.

DICKE, M., M. W. SABELIS, J. TAKABAYASHI, J. BRUIN, & M. A. POSTHUMUS, 1990 - *J. Chem. Ecol.* **16**, 3091-3118.

GEERVLIET, J.B.F. & J. BRODEUR, 1992 - *Med. Fac. Landbouww. Univ. Gent* **57/2b**, 547-550.

KAROWE, D.N. & L.M. SCHOONHOVEN, 1992 - *Entomol. Exp. Appl.* **62**, 241-251.

LAING, J.E. & D.B. LEVIN, 1982 - *Biocontr. & Inform.* **3**, 7-23.

LENTEREN, J.C. VAN, 1986 - In: J.K. Waage & D.J. Greathead (Eds.). *Insect parasitoids* 341-374. London: Academic.

LEWIS, W.J., L.E.M. VET, TUMLINSON, J.H., VAN LENTEREN, J.C. & D.R. PAPAJ, 1990 - *Envir. Entomol.* **19**, 1183-1193.

NEALIS, V.G., R.E. JONES & W.G. WELLINGTON, 1984 - *Oecologia (Berlin)* **61**, 224-229.

PRICE, P.W., 1981 - In: D.A. Nordlund, R.L. Jones & W.J. Lewis (Eds.). *Semiochemicals: their role in pest control.* 251-271. Wiley & Sons, New York.

PRICE, P.W., C.E. BOUTON, P. GROSS, B.A. MCPHERON, J.N. THOMPSON & A.E. WEISS, 1980 *Annu. Rev. Ecol. Syst.* **11**, 41-65.

STEINBERG, S., M. DICKE, L.E.M. VET & R. WANNINGEN, 1992 - *Ent. Exp. Appl.* **63**, 163-175.

TURLINGS, T.C.J., J.H. TUMLINSON, F.J. ELLER & W.J. LEWIS, 1991 - *Ent. Exp. Appl.* **58**, 75-82.

TURLINGS, T.C.J., J.H. TUMLINSON, W.J. LEWIS, 1990 - *Science* **250**, 1251-1253.

VET, L.E.M. & J.J.M. VAN ALPHEN, 1985 - *Oikos* **44**, 478-486.

VET, L.E.M. & M. DICKE, 1992 - *Annu. Rev. Entomol.* **37**, 141-172.

VET, L.E.M. & A.W. GROENEWOLD, 1990 - *J. Chem. Ecol.* **16**, 3119-3135.

VET, L.E.M., F.L. WÄCKERS & M. DICKE, 1991 - *Neth. J. Zool.* **41(2-3)**, 202-213.

VINSON, S.B., 1976 - *Annu. Rev. Entomol.* **21**, 109-134.

VINSON, S.B., 1981 - In: D.A. Nordlund, R.L. Jones & W.J. Lewis (Eds.). *Semiochemicals, their role in pest control* 51-77. Wiley & Sons, New York.

WÄCKERS, F.L. & W.J. LEWIS, 1993 - *Biol. Control* (in press).

WESELOH, R.M., 1981 - In: D.A. Nordlund, R.L. Jones & W.J. Lewis (Eds.). *Semiochemicals, their role in pest control* 79-95. Wiley & Sons, New York.

SYSTEMIC PRODUCTION OF HERBIVORE-INDUCED SYNOMONES BY LIMA BEAN PLANTS HELPS SOLVING A FORAGING PROBLEM OF THE HERBIVORE'S PREDATORS

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Key words:
tritrophic interactions, infochemicals, systemic effect, elicitor, Phytoseiidae

Summary
Upon infestation by herbivorous spider mites Lima bean plants start to produce a volatile herbivore-induced synomone that attracts phytoseiid mites that are predators of the spider mites. Predator-attracting infochemicals are produced systemically throughout the spider-mite infested plant. An elicitor has been extracted that is transported from the spider-mite infested leaves to uninfested leaves.

Introduction

Parasitoids and predators of herbivores have evolved and function in a multitrophic context. During foraging for herbivores the members of the third trophic level may use a wealth of stimuli. E.g. stimuli from the herbivore, from the food of the herbivore, from interactions between the herbivore and its food and from associated organisms such as microorganisms (Vinson 1976, Nordlund et al. 1981, Dicke 1988b). Thus, it may seem that natural enemies of herbivores have no problem in finding their victims. However, the usability of the stimuli mentioned above depends on two characteristics: their reliability and their detectability. It is argued that chemical stimuli originating from the herbivore have a high reliability in indicating herbivore presence, identity and accessibility, and that in contrast stimuli from the food of the herbivore have a relatively high detectability but a low reliability in indicating herbivore presence and identity (see Vet & Dicke 1992 for details). One of the solutions to this problem is available to the natural enemies in the form of so-called herbivore-induced synomones: plant chemicals whose production is induced by herbivory and that attract natural enemies of the herbivore. For example, upon infestation by two-spotted spider mites (*Tetranychus urticae*), Lima bean plants and cucumber plants emit large amounts of a blend of volatile chemicals that attract the predatory mite *Phytoseiulus persimilis*, which can exterminate *T. urticae* populations (Dicke & Sabelis 1988, Dicke et al. 1990ab). A similar phenomenon has been found for a tritrophic system consisting of corn plants, beet armyworm larvae (*Spodoptera exigua*) and the parasitic wasp *Cotesia marginiventris* (Turlings et al. 1990b, 1991).

Active role of plant in production of predator-attractants

The plant has an active role in this process and the chemicals that are emitted upon herbivory are not emitted upon artificial damage or only in minute quantities (Dicke

et al. 1990ab, Turlings et al. 1990b, 1991). Moreover, the herbivore-induced synomones seem to be rather specific: the natural enemies discriminate between different plant-herbivore combinations (Sabelis & Van de Baan 1983, Dicke 1988a, Dicke & Groeneveld 1986, Sabelis & Dicke 1985, Turlings et al. 1990a) and chemical differences between blends emitted by different plant-herbivore combinations have also been recorded (Dicke et al. 1990b, Takabayashi et al. 1991b). The exogenous elicitor triggering the plant response at the site of herbivory might be a component of herbivore saliva (Gäbler et al. 1992, Boland et al. 1992, Turlings & Tumlinson 1992).

The active role of the plant in the production of these volatile infochemicals has been inferred from two observations: the plant has a more pronounced influence on the composition of the chemical blend than the herbivore (Takabayashi et al. 1991b) and, more importantly, the production of volatile infochemicals that atttract natural enemies of herbivores is not restricted to the infested plant parts but occurs systemically throughout the plant (Dicke et al. 1990b, Turlings & Tumlinson 1992).

The systemic production of natural-enemy attracting infochemicals may add to their detectability. It implies that the source of the odour plume is not only larger than the herbivore itself, but that it is also larger than the infested plant part. The magnitude of this effect is dependent on the relative contribution of synomone production in uninfested leaves compared to that in the infested leaves themselves.

Systemic plant responses and endogenous elicitors

For several plant-herbivore interactions herbivore-induced systemic responses that affect *herbivore performance directly* are known to be mediated by *endogenous* elicitors that are transported from the infested site to the uninfested leaves (Bowles 1990b, Malamy et al. 1990, Métreaux et al. 1990, Ryan & Farmer 1991). The first evidence for the existence of an endogenous elicitor that mediates a systemic response that affects *natural enemy foraging behavior* has been reported recently.

For a tritrophic system consisting of Lima bean plants, the spider mite *T. urticae* and the predatory mite *Phytoseiulus persimilis* it was shown that uninfested leaves of a partially infested plant are more attractive to predatory mites than uninfested leaves of an *un*infested plant (Dicke et al. 1990b). This phenomenon can be explained by both the transport of an elicitor from the infested to the uninfested leaves, or the transmission of an electric signal (Wildon et al. 1989). Takabayashi et al. (1991a) added evidence in favour of the elicitor. They demonstrated for the same tritrophic system that uninfested Lima bean leaves that had been placed on wet cotton wool on which *in*fested Lima leaves had been present for the previous 7 days emit two known herbivore-induced synomone components in larger amounts than Lima bean leaves that had been placed on wet cotton wool on which *unin*fested Lima bean leaves had been present for the previous 7 days. These data may be explained by the presence of an elicitor that is in the wet cotton wool after the presence of the infested leaves. However, the data can theoretically also be explained by adsorption of the chemicals to the tray or the wet cotton wool and subsequently to the uninfested leaves.

The most elegant evidence for the existence of the endogenous elicitor in this tritrophic system has been recently presented (Dicke et al. 1993). Twenty Lima bean leaves were each placed with their petiole in water in a vial. Each leaf was infested

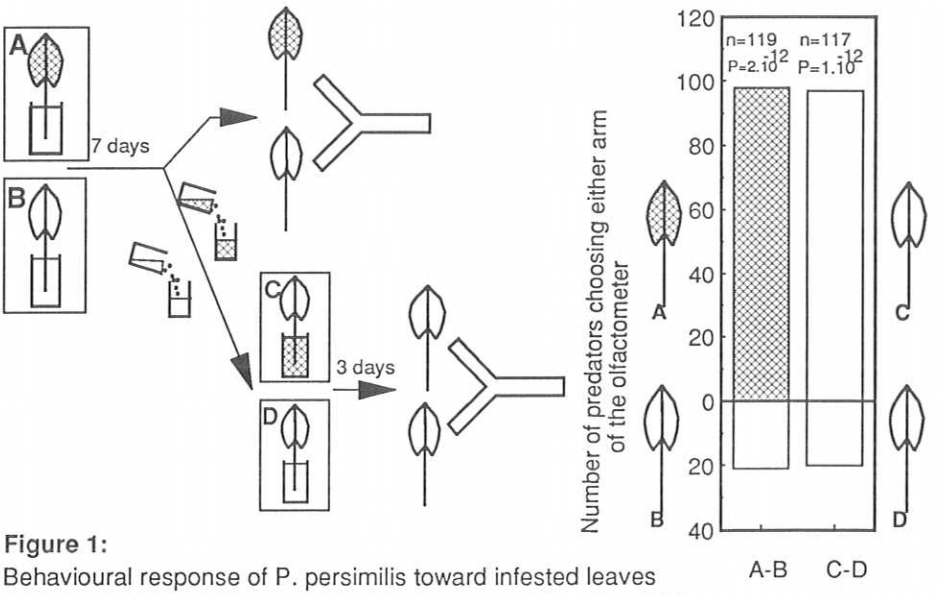

Figure 1:
Behavioural response of P. persimilis toward infested leaves or uninfested leaves that have been incubated in water in which infested leaves had been standing for the previous 7 days.

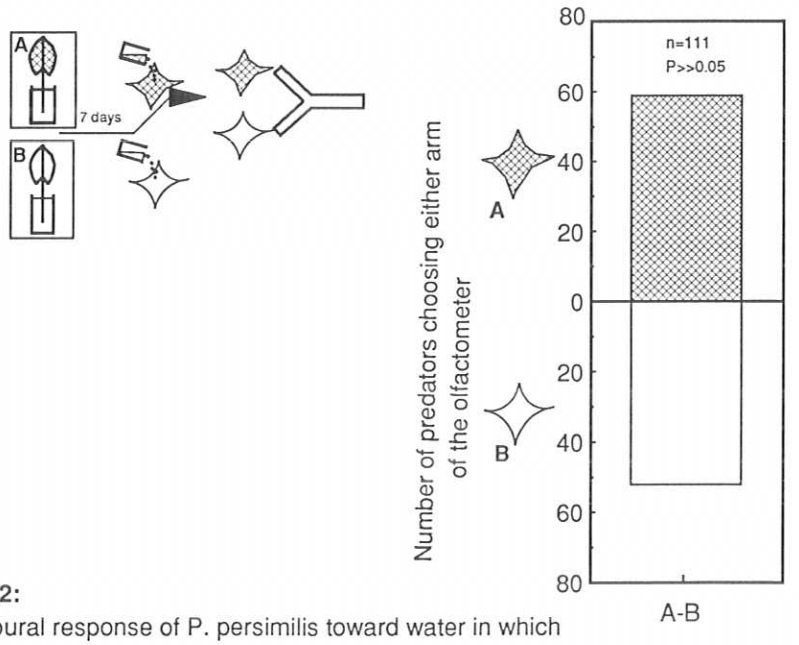

Figure 2:
Behavioural response of P. persimilis toward water in which infested or unifested leaves had been standing for 7 days Water was poured on filter paper.

with 50 adult spider mites. All vials were placed in a plastic cage. After 7 days the water was transferred to new vials and *un*infested Lima bean leaves were incubated in the water. The vials with leaves were now placed in a different cage. As a control we followed the same procedure but starting with uninfested instead of infested leaves, and using two different cages. The leaves were incubated in the water for 3 days and then used as odour source in a Y-tube olfactometer. The tubes with water were not used in the olfactometer. See Figure 1 for the procedures. The results of the experiment show that 20 leaves that had been infested by spider mites for 7 days are much more attractive than uninfested leaves. This confirms previous data (Sabelis & Van de Baan 1983). Moreover, it is remarkable that the predatory mites similarly prefer uninfested leaves that have been incubated for 3 days in water in which infested leaves had been standing for the previous 7 days (Figure 1). When the water in which the uninfested leaves had been incubated for 3 days was tested as an odour source in the Y-tube olfactometer the predators were not attracted (Figure 2). This demonstrates that the uninfested leaves are not attractive because they have absorbed a predator attractant from the water. Thus, the only explanation for the observed phenomenon is that an endogenous elicitor has been transported from the infested leaves to the uninfested leaves where it induced the production of the predator attractants.

Effects of endogenous elicitors

The elicitor may either induce this production directly or through activation of another step in a reaction chain. The exact mechanism of induction of synomone production remains to be elucidated. To do so, an important step is to identify the chemical structure of the currently demonstrated elicitor. Elicitors that are involved in the production of direct defenses that affect herbivore performance through inducing the production of digestibility reducers or phytoalexins have been reported for many systems. Among the identified signaling compounds are many oligosaccharides and other compounds such as a polypeptide, arachidonic acid, abscisic acid, methyl jasmonate, salicylic acid and inositol phosphates (Malamy et al. 1990, Métreaux et al. 1990, Ryan and Farmer 1991, Pearce et al. 1991). However, the functioning and recognition of these elicitors and thus their role in activation of plant genes has not yet been elucidated (Bowles 1990a, Ryan & Farmer 1991).

Sources of information for predatory mites

It is clear from our data that uninfested Lima bean leaves that have been exposed to an elicitor originating from spider-mite infested Lima bean leaves are highly attractive to predatory mites. This indicates that the systemic response may add to the attractiveness of a partially infested plant. Thus, predatory mites may perceive volatile infochemicals related to spider-mite infestations from three sources: (1) the spider mite-infested leaves (e.g. Sabelis & Van de Baan 1983), (2) the uninfested leaves of a spider-mite infested plant (Dicke et al. 1990b, this paper) and (3) the downwind neighbours of the spider-mite infested plant (Bruin et al. 1992). Current data indicate that the attractiveness of the uninfested leaves does not affect the ability of the predators to distinguish the infested leaves, which harbour the predator's prey (Dicke et al. 1993). This discrimination may be enabled by quantitative differences and in addition by qualitative differences. These aspects will be the subject of future investigations.

References

Boland, W.; Feng, Z.; Donath, J.; Gäbler, A., 1992. Are Acyclic C_{11} and C_{16} homoterpenes plant volatiles indicating herbivory? Naturwisschenschaften 79: 368-371.

Bowles, D., 1990a. Defense-related proteins in higher plants. Annu. Rev. Biochem. 59: 873-907.

Bowles, D., 1990b. Signals in the wounded plant. Nature 343: 314-315.

Bruin, J., Dicke, M. & Sabelis, M.W. 1992. Plants are better protected against spider mites after exposure to volatiles from infested conspecifics. Experientia 48: 525-529.

Dicke, M., 1988a. Prey preference of the phytoseiid mite *Typhlodromus pyri*: 1. Response to volatile kairomones. Exp. Appl. Acarol. 4: 1-13.

Dicke, M. 1988b. Microbial allelochemicals affecting the behaviour of Insects, Mites, Nematodes and Protozoa in different trophic levels. In: P. Barbosa & K. Letourneau (eds.) Novel Aspects of Insect Plant Interactions. John Wiley and Sons, New York, pp. 125-163.

Dicke, M.; Groeneveld, A., 1986. Hierarchical structure in kairomone preference of the predatory mite *Amblyseius potentillae*: dietary component indispensable for diapause induction affects prey location behaviour. Ecol. Entomol. 11: 131-138.

Dicke, M.; Sabelis, M.W., 1988. How plants obtain predatory mites as bodyguards. Neth. J. Zool. 38: 148-165.

Dicke, M., Baarlen, P. van, Wessels, R. & Dijkman, H. 1993. Herbivory induces systemic production of plant volatiles that attract the herbivores' predators: extraction of elicitor that serves as within-plant signal. J. Chem. Ecol. (submitted).

Dicke, M.; Beek, T.A. van; Posthumus, M.A.; Ben Dom, N.; Bokhoven, H. van; Groot, Æ. de, 1990a. Isolation and identification of volatile kairomone that affects acarine predator-prey interactions. Involvement of host plant in its production. J. Chem. Ecol. 16: 381-396.

Dicke, M.; Sabelis, M.W.; Takabayashi, J.; Bruin, J.; Posthumus, M.A., 1990b. Plant strategies of manipulating predator-prey interactions through allelochemicals: prospects for application in pest control. J. Chem. Ecol. 16: 3091-3118.

Gäbler, A.; Boland, W.; Preiss, U.; Simon, H. 1991. Stereochemical studies on homoterpene biosynthesis in higher plants; Mechanistic, phylogenetic and ecological aspects. Helvetica Chimica Acta 74: 1773-1789.

Malamy, J.; Carr, J.P.; Klessig, D.F.; Raskin, I. 1990. Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. Science 250: 1002-1004.

Métraux, J.P.; Signer, H.; Ryals, J.; Ward, E.; Wyss-Benz, M.; Gaudin, J.; Raschdorf, K.; Schmid, E.; Blum, W.; Inverardi, B. 1990. Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. Science 250: 1004-1006.

Nordlund, D.A.; Jones, R.L.; Lewis, W.J. (eds.), 1981. Semiochemicals, their role in pest control. Wiley & Sons, New York, 306 pp.

Pearce, G.; Strydom, D.; Johnson, S.; Ryan, C.A.;, 1991. A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. Science 253: 895-898.

Ryan, C.A.; Farmer, E.E., 1991. Oligosaccharide signals in plants: a current

assessment. Annu. Rev. Plant Physiol. Mol. Biol. 42: 651-674.

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

Sabelis, M.W. & Van de Baan, H.E., 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.

Takabayashi, J.; Dicke, M.; Posthumus, M.A., 1991a. Induction of indirect defence against spider-mites in uninfested lima bean leaves. Phytochem. 30: 1459-1462.

Takabayashi, J.; Dicke, M.; Posthumus, M.A., 1991b. Variation in composition of predator-attracting allelochemicals emitted by herbivore-infested plants: relative influence of plant and herbivore. Chemoecology 2: 1-6.

Turlings, T.C.J.; Scheepmaker, J.W.A.; Vet, L.E.M.; Tumlinson, J.H.; Lewis, W.J., 1990a. How contact foraging experiences affect preferences for host-related odors in the larval parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera:Braconidae). J. Chem. Ecol. 16: 1577-1589.

Turlings, T.C.J.; Tumlinson, J.H.; Lewis, W.J., 1990b. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250: 1251-1253.

Turlings, T.C.J.; Tumlinson, J.H.; Eller, F.J.; Lewis, W.J., 1991. Larval-damaged plants: source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the micro-habitat of its hosts. Entomol. exp. appl. 58: 75-82.

Turlings, T.C.J. & Tumlinson, J.H. 1992. Systemic release of chemical signals by herbivore-injured corn. Proc. Natl. Acad. Sci. USA 89: 8399-8402.

Vet, L.E.M. & M. Dicke, 1992. Ecology of infochemical use by natural enemies in a tritrophic context. Annu. Rev. Entomol. 37: 141-172.

Vinson, S.B., 1976. Host selection by insect parasitoids. Annu. Rev. Entomol. 21: 109-134.

Wildon, D.C.; Doherty, H.M.; Eagles, G.; Bowles, D.J.; Thain, J.F., 1989. Systemic responses arising from localized heat stimuli intomato plants. Annals of Botany 64: 691-695.

EFFECTS OF FLOWER COLOUR OF *CHRYSANTHEMUM* ON HOST PLANT RESISTANCE TO THE WESTERN FLOWER THRIPS (*Frankliniella occidentalis* Pergande) IN NO-CHOICE SITUATIONS

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Key words: *Frankliniella occidentalis*, *Dendranthema grandiflora*, flower colour, host plant resistance.

Summary

In 4 different no-choice experiments chrysanthemum plants of 11 colour mutants of the Reagan cultivar were tested on their resistance to thrips. The effects of thrips depended on the developmental stage of the plants and not on the flower colour.

INTRODUCTION

Frankliniella occidentalis Pergande is captured mostly in traps which have bright colours with wavelengths between 450 and 650 nm. Especially yellow and light blue are attractive (Brødsgaard 1989, Gillespie and Vernon 1990). In correspondence, yellow coloured flowers are more attractive, but the question whether colourpigments of flowers have an effect on antixenosis or antibiosis of the hostplant has not been investigated. The experiments described here deal with the effects of thrips on plants and the growth of the population of the insects on plants with a different set of colourpigments in no-choice situations.

MATERIALS AND METHODS

In the experiments 11 colour mutants of the cultivar Reagan were tested. The mutants are: White (- anthocyanin and - carotenoid), yellow (-,+), pink (+,-) and red (+,+). Of each colour group at least 2 mutants were tested.
In the first three experiments (A, B and C), 5 out of 10 plants of 9 colour mutants, were infested with 20 adult ♀♀ thrips. Minimum temp. was 23° and R.H. was kept on a minimum of 70%. The other five plants were control plants. Plants were kept separately in cages and exposed to natural short daylength to induce flower buds. Before the experiments started they were treated with HCN to kill all insects and mites.
A In week 5, before flower buds were visible, 20 adult ♀♀ thrips were released. After 21 days the diameter of the largest flower bud was measured (ϕbud). 10 leaves evenly spaced from the top to the bottom were sampled and the thrips damaged leaf area (TDLA) was measured in mm^2 per leaf with an image analysis system (TCL-image).
B In week 8, plants with the same history, but with small flower buds were infested with 20 adult ♀♀ thrips. After 21 days TDLA and the number of thrips nymphae (N_n) in the flowers was measured.

C From week 10 on, plants were infested with thrips as soon as the first row of anthers in the florescence were ripe (developmental stage DS 1). After 12 days four flowers were sampled and N_n was counted.
D In a separate experiment, individual flowers from 7 colour mutants at DS 1 were taken, put in a cage, infested with 10 adult ♀♀ thrips and kept under controlled conditions. After 4, 8 and 12 days N_n in 6 flowers of each mutant was established.

RESULTS

In Table 1 relevant data of the four experiments are given. There are significant differences between genotypes, which is shown by means followed by different letters. The least resistant mutant is I, but this is fully explained by the earlier flowering, which is observed in this mutant. Both in Exp. A and B mutant I opened flowers one week earlier, thus providing a better substrate for the thrips to feed on. The correlation between ϕbud and N_n is R = 0.847 *** and between N_n and TDLA is R = -0.759 **. Measurements of other plant characteristics as plant length and total leaf area did not differ between mutants.

CONCLUSIONS

In no-choice situations in chrysanthemum plants, the amount of damage caused by the Western Flower Thrips and the thrips development, are not associated with the flower colour but they are strongly correlated with the developmental stage of the plants.

REFERENCES

GILLESPIE, D.R. & R.S. VERNON, 1990 - Trap catch of western flower thrips (Thysanoptera: Thripidae) as effected by color and heigth of sticky traps in mature greenhouse cucumber crops. *J. Econ. Entomol.* **83**, 971-975.
BRODSGAARD, H.F.,1989 - Coloured sticky traps for *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae) in glasshouses. *J.Appl.Entomol.* **107**, 136-140.

Table 1. Results of 4 experiments with colour mutants of chrysanthemum on host plant resistance to the thrips, *Frankliniella occidentalis*.

Mutant	Experiment A		Experiment B		Experiment C	Experiment D		
	TDLA	ϕbud	TDLA	N_n	N_n	N_4	N_8	N_{12}
I -,-	4.1 b	4.8 b	16a	217 b	302 b	-	-	-
II -,-	3.8ab	4.0ab	179 bc	13a	274 b	41a	353	207
III -,+	4.9 b	4.6ab	153 b	17a	243ab	48ab	326	258
IV -,+	3.6ab	4.6ab	111 b	30a	269ab	58 b	336	215
V +,-	4.3 b	5.4 b	119 b	24a	258ab	-	-	-
VI +,-	1.7a	3.6ab	179 bcd	9a	191ab	54 b	323	156
VII +,-	2.9ab	3.2a	183 bcd	11a	147a	50ab	270	250
VIII +,-	3.9ab	3.8ab	226 cd	19a	227ab	-	-	-
IX +,+	3.6ab	3.4ab	250 d	10a	-	-	-	-
X +,+	-	-	-	-	-	55ab	306	220
XI +,+	-	-	-	-	-	44ab	286	210

HOST SELECTION IN *COTESIA FLAVIPES*, PARASITOID OF TROPICAL STEMBORERS

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Key words: Hymenoptera, Braconidae, Lepidoptera, Pyralidae, Noctuidae, foraging behaviour

SUMMARY

The host-selection behaviour of *Cotesia flavipes*, a parasitoid of graminaceous stemborer larvae, was investigated in a Y-tube setup and through direct observations of the behaviour on maize plants. Dual choice tests in the Y-tube setup revealed that maize plants infested with two *Chilo partellus* or *Busseola fusca* larvae were attractive to females of *Cotesia flavipes*. The parasitoid did not have a preference for either species. No differences were found in the behaviour of *C. flavipes* on maize plants infested with *C. partellus* or *B. fusca*. *C. flavipes* appears not to be specific in regard to the species she will attack on a particular plant species.

INTRODUCTION

For biological control to be a reliable and efficient method, insight is needed in the foraging behaviour of the natural enemy used. The ability of parasitoids to locate and attack hosts is a key determinant of how well a given parasitoid population performs, thus variability in this host-location or host-selection can be a major source of inconsistent results in biological control with parasitoids (Lewis et al., 1990). A parasitoid can have a preference for a particular herbivore or plant species. This preference can be innate or modified through adult learning (Vet & Dicke 1992). The manipulation of a preference for a particular host species or plant species has far reaching implications for biogical control. Turlings et al. (1990) not only showed that *Cotesia marginiventris* can learn to distinguish between the odours of two different plant species fed upon by the same host species, but also between the odours of two different host species feeding on the same plant.

In this study we investigated the host-selection behaviour of *Cotesia flavipes* (Cam.) (Hymenoptera: Braconidae). *C. flavipes* is a gregarious larval parasitoid of various noctuid and pyralid stemborers and has been extensively used in classical biological control programmes against stemborers in various graminaceous crops (refs. in: Polaszek & Walker, 1991). This parasitoid is a candidate for biological control against *Chilo partellus* (Swinhoe), the major pest in maize and sorghum in Africa. Another stemborer of significant economic importance in these crops is *Busseola fusca*

(Hampson). In contrast to the pyralid *C. partellus*, from which *C. flavipes* was collected in its native area, the noctuid *B. fusca* is a new host for *C. flavipes*. In laboratory experiments it was shown that *B. fusca* is an unsuitable host for *C. flavipes*: the encapsulation rates of the eggs of *C. flavipes* are very high (Overholt & Ngi-Song, unpublished data).

In this study a comparison was made of the searching behaviour of *C. flavipes* on and towards maize plants infested with *C. partellus* or *B. fusca*.

MATERIAL & METHODS

Hosts The hosts used in the experiments were fourth instar larvae of *C. partellus* and *B. fusca* reared on artificial diet, according to the method of Ochieng et al. (1985).

Parasitoids *C. flavipes* were reared on *C. partellus* larvae feeding on maize stems or artificial diet. The cocoons of the wasps were collected after 10-11 days and stored (at 25 °C) in a glass vial until emergence. Prior to an experiment the naive females (mated, 1-2 days old) were individually transferred to a small vial.

Experimental procedures

Treatment of Plants Uninfested maize plants (tassel - 70-110 cm) obtained from a farmer's field were used as a standard clean maize plant. A Plant-Host-Complex (PHC) was obtained by introducing two stemborer larvae into holes bored horizontally in the stem, spaced 10 cm apart, with the first hole 5 cm from soil level. The larvae were introduced into the plant 18 h prior to the first experiment. During this time the larvae bored a small tunnel in the stem and most of them pushed some frass out of the artificially bored entrance hole.
The treatments used in the experiments were:
PHC-FUSCA maize plant infested with two fourth-instar *B. fusca* larvae.
PHC-PART maize plant infested with two fourth-instar *C. partellus* larvae.
CONTROL A standard uninfested maize plant.

Behaviour on plant The foraging behaviour of the parasitoids on maize plants took place in a room completely covered with white sheets to obtain a contrasting background for the parasitoids. A treated maize plant (PHC-FUSCA, PHC-PART or CONTROL) was placed on a metal stand. A female wasp was released on the stem 25 cm from soil level, thus 10 cm above the top hole on infested plants. The behaviour of the parasitoid was observed continuously and recorded with a portable computer programmed as an eventrecorder.
The following parameters were recorded:
Behaviour: Stand, Walk, Fly, Preen and Examine frass or entrance hole.
Location: Stem (inside or outside), Entrance Hole, Whorl, Leaf
Experiments were terminated after 90 minutes or when the parasitoid left the plant. After each replicate the tested plant was removed and replaced by a new one. If the parasitoid entered the stem, the larva in that hole was removed, introduced into a vial containing artificial diet and a few days later dissected to check if it was parasitized. For wasps that

did not come out of a hole until the observation ended, the stem was opened to check if the wasp was dead or alive.

Y-tube setup The response of the parasitoids towards volatiles emanating from maize plants was investigated in a Y-tube olfactometer. For a detailed description of the olfactometer, see Sabelis & van de Baan (1983) and Steinberg et al. (1992). The major modification in this setup was the odour source container. It consisted of a Plexiglas cage (30x30x122 cm), large enough to contain a medium sized maize plant.
Parasitoids were individually introduced in the Y-tube and given a maximum of 5 min to make a choice for one of the arms. When the wasp stayed more than 15 sec beyond the finish line (8 cm from intersection) it was recorded as a choice. The odour sources were exchanged after testing 5 parasitoids to rule out any asymmetric aspect of the setup. The three dual choice tests, which were always tested on the same day, consisted of:

A) PHC-FUSCA versus CONTROL
B) PHC-PART versus CONTROL
C) PHC-FUSCA versus PHC-PART

The tests were performed at 23-26 °C, RH 65-75% and light intensity 350-440 lux. The choice of the parasitoids (n=60 per combination) was analyzed with a Chi^2 test (P = 0.05).

RESULTS & DISCUSSION

Behaviour on plant

The mean foraging time of *C. flavipes* on infested plants was significantly longer than on uninfested maize plants. There was no difference in the searching time on *C. partellus* or *B.fusca* infested maize plants (Table 1). After release on the stem the parasitoids immediately started walking, sometimes interrupted by periods of standing or preening. On infested plants a significantly higher fraction of the parasitoids visited the lower part of the stem (Table 1) and most wasps found the spot of infestation within 5 min. The entrance hole of the tunnel was usually filled with frass pushed out of the tunnel by the feeding stemborer larva. After contact with the frass the female started antennating the frass and immediately tried to enter the tunnel by crawling through the frass. The time spent inside the hole varied considerably (3 - 5209 sec). Sometimes the parasitoid re-entered the tunnel several times, probably in case it could not reach the larva. Because a female *C. flavipes* needs only a few seconds to parasitize the host (as seen in the culture), the time spent inside the tunnel is probably dependent on the location of the larva and the amount of frass in the tunnel. Parasitization rates of the larvae were low (23%-33%). This is probably caused by the high mortality rate of the parasitoids attacking a larva. The stemborer larvae defend themselves by spitting and biting upon contact with the parasitoid, especially during oviposition of the parasitoid. 30-50% of the parasitoids entering the tunnel were killed in this way. However some parasitoids were able to parasitize the larvae before being killed. There were no differences found in the behavioural parameters of *C. flavipes* attacking either *C. partellus* or *B. fusca* (Table 1).

Y-tube experiments

Parameter	Treatment of maize plant		
	Chilo partellus	*Busseola fusca*	Uninfested
Mean residence time on plant (sec)[1]	4782a (n=19)	4567a (n=13)	1670b (n=20)
Percentage visiting lower part of plant	61a (n=23)	86a (n=22)	25b (n=20)
Latency time location entrance tunnel[2]	418a (n=13)	247a (n=16)	
Mean time spent inside stem (sec)[3]	2138a (n=9)	1670a (n=8)	
Percentage killed inside stem[2]	31a (n=13)	50a (n=16)	
Parasitization percentage[2]	23a (n=13)	33a (n=16)	

Table 1
Behaviour of C. flavipes females on uninfested maize plants and maize plants artificially infested with two fourth-instar larvae of C. partellus or B. fusca. Significant differences are indicated by different letters. Means tested with Mann-Whitney U test, ratios with G-test (α = 0.05). [1]Excluding wasps that died inside stem. [2]Of those wasps that located and entered tunnel. [3]Of those wasps that did not die inside tunnel.

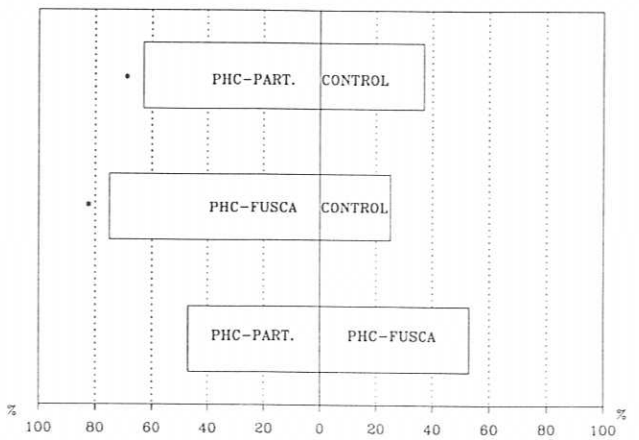

Figure 1
Responses of C. flavipes females in Y-tube olfactometer dual choice tests. CONTROL = uninfested maize plant; PHC-PART. = maize plant artificially infested with two fourth-instar larvae of C. partellus ; PHC-FUSCA = maize plant artificially infested with two fourth-instar larvae of B. fusca. An asterisk indicates a significant preference for an odour source (α = 0.05).

The previous experiment showed that once a *C. flavipes* female has landed on an infested plant, she does not seem to make a distinction between different stemborer species feeding upon the plant. To test wether *C. flavipes* makes a distinction in an earlier phase of the host-location process, the response to volatile cues was tested in a Y-tube olfactometer.
The results are summarized in figure 2. Maize plants, wether infested by *C. partellus* or *B. fusca* attracted the majority of parasitoids when offered versus a control plant. In a dual choice test *C. flavipes* did not make a distinction between *C. partellus* and *B. fusca* infested maize plants.

The host-location process can be divided in: location of an infested plant, location of the host on the plant and acceptance of the host. The experiments showed that in the different phases of the host-location process, *C. flavipes* does not make a distinction between stimuli released from maize plants infested with *C. partellus* or *B. fusca*. The Y-tube experiments showed that the parasitoids were attracted to volatiles emanating from infested maize plants, irrespective of the stemborer species feeding upon the plant. The direct observations of the foraging behaviour of *C. flavipes* on infested plants revealed that both stemborer species were attacked and accepted for oviposition. It seems that *C. flavipes* is not specific with regards to the host species it attacks on a particular plant species. The volatile and contact stimuli released by stemboring larvae, feeding upon a maize plant, seem to be very similar and the parasitoid probably attacks all the species encountered in the stem of the plant.

C. flavipes has a short lifespan and small eggload: a female parasitoid lives only for a few days and has around 150 eggs available for ovipostion (Overholt & Potting, unpublished data). A female allocates around 30-40 eggs per host, so during her life a female probably parasitizes a few hosts. Overholt & Ngi-Song (unpublished data) showed that the pararsitoids eggs were encapsulated by *B. fusca*. Although *B. fusca* is unsuitable for the developent of the parasitoid, it is accepted for oviposition. It seems in fitness terms an expensive mistake for a time- and egglimited parasitoid, to allocate time and progeny to an unsuitable host. However *C. flavipes* and *B. fusca* have not coevolved with each other and it remains to be investigated if *C. flavipes* rejects unsuitable sympatric host species.

REFERENCES

LEWIS, W.J., VET, L.E.M., TUMLINSON, J.H., LENTEREN, J.C. van & D.R. PAPAJ, 1990 - Variations in parasitoid foraging behavior: essential element of sound biological control theory. *Environ. Entomol.* **19**: 1183-1193.

OCHIENG, R.S., ONYANGO, F.O & M.D.O. BUNGU, 1985 - Improvement of techniques for mass-culture of *Chilo partellus* (Swinhoe). *Ins. Sci. Appl.* **6**: 425-428.

POLASZEK, A. & A.K. WALKER, 1991 - The *Cotesia flavipes* species-complex: parasitoids of cereal stemborers in the tropics. *REDIA* **74**: 335-341.

SABELIS, M.W. & 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.

STEINBERG, S., DICKE, M., VET, L.E.M. & R. WANNINGEN, 1992 - Response of the braconid *Cotesia (=Apanteles) glomerata* to volatile infochemicals: effect of bioassay set-up, parasitoid age and experience and barometric flux. *Entomol. exp. appl.* **63**: 163-175.

TURLINGS, T.C.J., SCHEEPMAKER, J.W.A., VET, L.E.M., TUMLINSON, J.H. & W.J. LEWIS, 1990 - How contact foraging experiences affect preferences for host-related odors in the larval parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). *J. Chem. Ecol.* **16**: 1577-1589.

VET, L.E.M. & M. DICKE, 1992 - Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* **37**: 141-172.

VINSON, S.B., 1976 - Host selection by insect parasitoids. *Annu. Rev. Entomol.* **21**: 109-134.

THE LEAVING TENDENCY OF THE PARASITOID *ENCARSIA FORMOSA* FORAGING FOR WHITEFLY ON TOMATO LEAFLETS

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Key words:
parasitoids, patch time allocation, giving up time, residence time, proportional hazards.

Summary
The effect of experiences, such as contact with honeydew, antennal or ovipositorial rejections and ovipositions, and temperature, on the leaving tendency of individual *Encarsia formosa* female parasitoids on tomato leaflets have been studied. Behavioural records were analysed by means of the proportional hazards model. The median time from being placed on the leaflet or, if occurred, from the latest encounter until leaving was 18.6 minutes. The effect of temperature, ranging from 20 to 30°C, was negligible. The presence of honeydew and the first oviposition in unparasitized hosts decreased the leaving tendency, increasing the time from being placed on the leaflet or, if occurred, from the latest encounter until leaving. Encounters with parasitized hosts did not affect the leaving tendency; as a result they did increase the total residence time.

INTRODUCTION

In a parasitoid-host interaction it is necessary for each parasitoid to search for hosts to reproduce. The number of offspring realized per parasitoid depends on searching efficiency and on how the parasitoid reacts to experiences in the host patch, for instance ovipositions or rejections. In order to maximize its fitness a parasitoid should react to certain experiences on a patch in a functional way. Waage (1979) predicts that ovipositions in unparasitized host should increase the time spent in a patch since the latest oviposition (giving up time). This effect is assumed to be deterministic. Encounters with parasitized hosts, however, are expected to give rise to a decrease.

Here, we study the influences of different experiences and temperature on the leaving tendency of the whitefly parasitoid *E. formosa* on a tomato leaflet, i.e. the probability per unit time to leave the experimental leaflet. *E. formosa* Gahan (Hymenoptera: Aphelinidae) is a synovigenic, solitary larval parasitoid of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). The parasitoid moves from leaf(let) to leaf(let) by flying or hopping. Once on the leaf it starts walking and drumming the leaf with its antennae. Upper leaf sides may be covered by honeydew produced by hosts in higher leaf layers.

Whitefly immatures are sessile and develop on the lower leaf side from the egg stage to four successive larval stages (L1-L4), a prepupa and a pupa stage (van Roermund & van Lenteren, 1992). The L3, L4 and prepupa are preferred by *E. formosa* for oviposition. On encounter, a host can be rejected after an inspection with the antennae (antennal rejection) or can be rejected or accepted after an insertion of the ovipositor (ovipositorial rejection, oviposition or host feeding) (van Lenteren et al., 1980). About ten days after oviposition the parasitoid immature pupates in the host pupa, which then becomes black.

Experiments were carried out in which individual parasitoids were observed continuously on leaflets. The regression model that we applied is a special application of the proportional hazards model (Cox, 1972; Kalbfleisch and Prentice, 1980). This model incorporates factors which are expected to be relevant from a functional or physiological point of view. The factors (also called covariates) considered here are presence of honeydew, number of antennal or ovipositorial rejections and ovipositions, and temperature. The leaving tendency was simultaneously estimated with the effects of the afore mentioned factors. The significance of these effects was then tested. In this way the relative importance of the factors considered in the process of decision-making can be assessed quantitatively.

The model was chosen to analyse the behavioural data because it is a stochastic model, it makes no assumptions about how probabilities per time unit change with time, it is easily adapted to different situations and censored data are handled accurately.

MATERIAL AND METHODS

Tomato plants. Tomato plants (*Lycopersicon esculentum* var. 'Moneymaker') were grown in a greenhouse compartment at 20-24°C, 16 hour daylength and 70% RH. Plants were used when 4-6 weeks old and about 60 cm in height. Leaflets on which observations were carried out were 22.5 $cm^2 \pm 0.6SE$ (n=110).

Greenhouse whitefly. Greenhouse whiteflies were reared on tomato var. 'Moneymaker' in a greenhouse compartment at 24°C, 16 hour daylength and 50% RH. The whitefly rearing has been kept on tomato for over 15 years. A film of honeydew with small pieces of exuviae was collected on upper sides of leaflets by placing an infested leaflet bearing $15.9 \pm 0.93SE$ (n=30) immatures (all stages) per leaf disk of 0.196 cm^2 slightly above a clean leaflet in a holder for 24 hours at 22°C.

Parasitoids. Parasitoids were delivered as black pupae on paper cards by Koppert B.V., (Berkel en Rodenrijs, the Netherlands). For each observation a naive female of *E. formosa*, not older than 24 hours was used. From at least 1 hour before the start of the observation parasitoids were kept separately in a polysacharid capsule (0.8 cm, 2.5 cm).

Experimental set-up. All replicates were carried out in a climate room at a constant temperature ($\pm$1°C) and 70 $\pm$5% RH on tomato leaflets. At the start of each replicate a single *E. formosa* female was introduced on a leaflet. Continuous observation started directly after the introduction of the parasitoid. The following behavioural components were recorded using the computer software package 'the Observer' (Noldus, 1991): a) searching, b) standing still/ eating honeydew/ preening, c) drumming the host with antennae, d) oviposition posture and e) host feeding. An observation stopped when the parasitoid flew from the leaflet or when it walked off

the petiole, which was only rarely observed. When no foraging activity occurred for more than 60 minutes, observation was also stopped.

Two types of experiments were conducted. In experiment I no hosts were present and in experiment II a varying number of unparasitized or parasitized hosts were present on the lower leaf side. Hosts were parasitized by conspecific females 1 to 7 hours and about ten days before observation. In experiment I the treatments were:

- 25°C, without honeydew, parasitoid introduced on upper leaf side (n=39)
- 25°C, without honeydew, parasitoid introduced on lower leaf side (n=45)
- 25°C, with honeydew and parasitoid introduced on upper leaf side (n=24).
- 20°C, without honeydew, parasitoid introduced on upper leaf side (n=29)
- 30°C, without honeydew, parasitoid introduced on upper leaf side (n=50)

In experiment II the ambient temperature was always 25°C, honeydew was absent and the parasitoid was introduced on the lower leaf side. As a control, the group of 45 replicates from experiment I was taken. The treatments were:

- 1 unparasitized L3/L4 larva (n=24)
- 4 unparasitized L3/L4 larvae (n=27)
- 1 recently-parasitized L3/L4 larva (n=43)
- 4 recently-parasitized L3/L4 larvae (n=43)
- 4 parasitized black hosts (n=41)
- 77 to 200 parasitized black hosts (n=23).

DESCRIPTION OF THE REGRESSION MODEL

The proportional hazards model is formulated in terms of the hazard rate, which is the probability per unit time that a certain event (a so-called failure) occurs, given that it has not occurred yet, since the latest renewal point (see below). The hazard rate can be considered as a tendency to perform a certain behaviour, here leaving a leaflet. When studying time periods until leaving, leaving a leaflet is a failure. The encounter with a host causes a censored observation of the searching time, because it is not known when the parasitoid would have left if the host had not been encountered. Renewal points occur here (only) at the times that hosts are encountered, because the searching process of *E. formosa* is clearly interrupted. It is assumed that parasitoids have a basic tendency to perform a certain behaviour (base line hazard), which is reset after certain renewal points. The observed hazard rate is assumed to be the product of the base line hazard and a factor that gives the joint effect of a set of p fixed covariates $z_1,....,z_p$. The covariates are for instance the intra-patch experiences, such as the number of ovipositions (with values 0,1,2, etc.) or the absence or presence of honeydew (with value 0 or 1 respectively). They are called fixed when they do not change during the time between two successive encounters. Then the general form of the model is:

$$(1) \qquad h(t;z) = h_0(t)\ \exp\{\sum_{i=1}^{p} \beta_i\ z_i\}$$

where $h(t;z)$ denotes the observed hazard rate, $h_0(t)$ the base line hazard, t the time since the latest renewal point and $\beta_1,....,\beta_p$ the relative contributions of the covariates $z_1,....,z_p$. The form of the base line hazard in time is left unspecified; $h_0(t)$ as well as

$\beta_1,....,\beta_p$ are estimated from the data by means of likelihood maximization. The test statistic is distributed as a χ^2 with p degrees of freedom. The name 'proportional hazards model' stems from the assumption that for different values of z_i the hazard rates $h(t;z)$ are proportional. If this assumption is justified will be checked during analysis (see Haccou and Hemerik, 1985; Kalbfleisch and Prentice, 1980 for further details).

The data used in the regression model were the observed time periods from being placed on the leaflet until the first encounter, between successive encounters, and from the last encounter until leaving the leaflet. When no encounters occur, this time period is equal to the residence time. During such a period, the parasitoid can either search for hosts, stand still, preen or eat honeydew. The tendency to leave the leaflet is given by equation (1) with 4 and 6 fixed covariates for experiment I and II respectively (see Table 1b).

RESULTS

The estimated leaving tendency and the effects of several covariates are given in Table 1. The leaving tendencies (base line hazards, in probability to leave per unit of time) of Experiment I and II were almost equal although in the last experiment the number of hosts ranged from 1 to 200 (Table 1a). The combined effect of all covariates is significant in both experiments (not shown).

A film of honeydew with small pieces of exuviae on the upper leaf side strongly reduces the leaving tendency of the parasitoid. The multiplication factor $\exp(\beta)$ is below 1, which results in a lower hazard rate $h(t,z)$ according to equation (1): time periods on leaflets with honeydew are much higher than on clean leaflets. The leaf side on which the parasitoid started and the temperature did not influence leaving tendency significantly on clean leaflets (Table 1b).

The cumulative base line hazards were approximately straight lines, so the leaving tendency remained constant over time. Then the fraction of parasitoids that remain on a leaflet over time (the survival function) follows a decreasing exponential distribution. Thus the median time period on a leaflet can be estimated by -ln(0.5) divided by the leaving tendency. This results in a median residence time of 1116 seconds (18.6 minutes) on clean tomato leaflets and 5978 seconds (99.6 minutes) on uninfested tomato leaflets containing large amounts of honeydew with small pieces of exuviae on the upper leaf side.

In experiment II the first oviposition in an unparasitized host strongly reduced the leaving tendency (Table 1b), resulting in an increase in the time period since the latest encounter until leaving to 4201 seconds (40 minutes). In a preliminary study the effect was tested for all realized ovipositions ranging from 0 to 4, but no clear difference was found for the effect after one or more ovipositions.

Time since being placed on the leaflet and encounters with parasitized hosts did not affect the leaving tendency significantly (Table 1b). Even encounters with black parasitized hosts did not affect the leaving tendency, although leaflets with 77 to 200 black hosts were tested. On these leaflets, the number of encounters was on average 53 (range 12-134). However, each encounter with hosts, whether or not parasitized, does increase the residence time on a leaflet, since renewal points were taken after each encounter and the time period since the latest encounter until leaving is not reduced.

Table 1a. Estimated leaving tendency (base line hazard in sec^{-1}) in experiment I and II.

Experiment I	$6.21*10^{-4}$
Experiment II	$7.32*10^{-4}$ +

+: estimated without censors due to encounters

Table 1b. Estimated effects (multiplication factor exp β) of covariates on the leaving tendency in experiment I and II and the value of the test statistic T.

	effect	T (df)
Experiment I		
introduction on upper leaf side	0.7788	1.23 (4)
honeydew on upper leaf side	0.1867	27.05 (4) *
temperature 20°C	0.9343	0.07 (4)
temperature 30°C	0.7027	2.37 (4)
Experiment II		
time since being placed on leaflet [1)]	1.0000	1.89 (6)
antennal rejection of recently-parasitized hosts	1.1351	3.73 (6)
antennal rejection of parasitized black hosts	0.9948	0.03 (6)
ovipositorial rejection of recently-parasitized hosts	0.7255	3.63 (6)
oviposition in recently-parasitized hosts	0.8422	1.01 (6)
oviposition in unparasitized hosts [2)]	0.4648	16.02 (6) *

*: $P < 0.05$; [1)]: effect given per second; [2)]: effect given when covariate is 0/1 for no/one or more events

DISCUSSION

Leaving a leaflet is clearly a stochastic process, characterized by a certain probability per unit of time. For *E. formosa* on tomato leaflets this probability is approximately constant over time and the median time period that the parasitoids remain on a leaflet is 18.6 minutes after landing or after their latest encounter with a host.

The first oviposition in an unparasitized host and the presence of a film of honeydew with small pieces of exuviae decrease the leaving tendency on tomato leaflets. This increases the time period since landing or since the latest encounter to a median of about 40 and 100 minutes respectively. The observed responses increase the likelihood of encountering hosts in a natural, clumped host distribution.

Honeydew is apparently associated with the presence of hosts. Van Vianen & van der Veire (1988) also observed an increase in time on the leaf after *E. formosa* discovered honeydew.

Haccou et al. (1991) and Hemerik et al. (in press) found a multiplication factor of 0.87 and 0.80 per oviposition on leaving tendency after ovipositions in unparasitized hosts for *Leptopilina heterotoma* and *L. clavipes* respectively. These parasitoids are mainly time-limited (Driessen & Hemerik, 1992). The effect for *E. formosa* is initially stronger, namely a multiplication factor of 0.46 for the first oviposition. However, for *E. formosa* every encounter was treated as a renewal point, because even a rejection is a clear interruption of the searching process, due to the relative long handling times compared to that of *L. heterotoma* and *L. clavipes*. The second, third and fourth oviposition give no additional effect. This can be explained, because *E. formosa* is a synovigenic parasitoid with an egg load of 8-10 mature eggs (van Vianen & van Lenteren, 1986), so once on a patch with hosts the number of mature eggs soon becomes a more critical limitation than time. The parasitoid should be more careful with its last eggs and time is no longer limited to search for other patches.

Encounters with parasitized hosts do not affect the leaving tendency of *E. formosa*, even though experiments were conducted in which more than 100 such encounters were realized. Although the leaving tendency does not change after such encounters, the residence time on a leaflet does increase. When the presence of parasitized hosts increases the probability of encountering unparasitized hosts, there is no need for the parasitoids to increase the leaving tendency after rejections when they are not time-limited. Under natural circumstances the presence of parasitized hosts can be a good indicator of unparasitized hosts, because not all unparasitized hosts on a leaflet are parasitized by one *E. formosa*. This is caused by the parasitoids' random walking pattern and limited residence time at low host densities and by egg-limitation at higher densities. Haccou et al. (1991) also found no effect of encounters with parasitized hosts on the leaving tendency of *Leptopilina heterotoma*. For *L. clavipes*, the effect was dependent on whether previous ovipositions had occurred (Hemerik et al., in press).

Each encounter with a host, whether or not parasitized, leads to an increase in total residence time of *E. formosa*. From these experiments it can be shown that the upper leaf side is important in determining the total time on a leaflet because on this side no encounters with hosts take place. The tendency to change from one leaf side to another is studied by van Roermund et al. (in press).

Temperature ranging from 20 to 30°C does not influence the leaving tendency. This tendency is apparently not affected by physiological processes, which are usually temperature dependent. Daily temperature fluctuations in these ranges are common and relatively fast. As temperature fluctuations do not influence the distribution of the sessile hosts, there is no advantage for the parasitoids to change its leaving tendency.

Several hypotheses have been proposed for patch-leaving mechanisms, such as (a) a patch is left after a fixed number of hosts is parasitized, (b) the parasitoids leave the patch after a fixed period of time and (c) the parasitoids leave after the oviposition or encounter rate falls below a certain threshold (Gibb, 1962; Krebs, 1973; Murdoch & Oaten, 1975; Waage, 1979). The patch-leaving behaviour of

E.formosa can be described by a stochastic mechanism and is neither a fixed number or fixed time mechanism. After each encounter, the time until leaving varies because it is driven by a basic tendency (probability per time) to leave the patch, which is decreased after the first oviposition in an unparasitized hosts and by the presence of honeydew.

An advantage of the proportional hazards model is that the outcome can be incorporated directly into simulation models. The estimated leaving tendency (and the tendency to change leaf sides) together with the significant effects of certain types of encounters with hosts and honeydew will be used as input in a stochastic simulation model of the foraging behaviour of *E. formosa* on a leaf(let) (van Roermund & van Lenteren, in prep.). This model simulates the functional response on a leaf(let) in a natural situation, where the parasitoid can fly to other leaves or leaflets.

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REFERENCES

Cox, D.R., 1972. Regression models and life tables. *Biometrics* 38, 67-77.

Driessen, G. & Hemerik, L., 1992. The time and egg budget of *Leptopilina clavipes*, a parasitoid of larval *Drosophila*. *Ecological Entomology* 17: 17-27.

Gibb, J.A., 1962. Tinbergen's hypothesis of the role of specific search images. *Ibis* 104, 106-111.

Haccou, P. & Hemerik, L., 1985. The influence of larval dispersal in the cinnabar moth (*Tyria jacobaeae*) on predation by the red wood ant (*Formica polyctena*): an analysis based on the proportional hazards model. *Journal of Animal Ecology* 54, 755-770.

Haccou, P., de Vlas, S.J., van Alphen, J.J.M. & Visser, M.E., 1991. Information processing by foragers: effects of intra-patch experience on the leaving tendency of *Leptopilina heterotoma*. *Journal of Animal Ecology* 60, 93-106.

Hemerik, L., Driessen, G. & Haccou, P., in press. Effects of intra-patch experiences on patch time, search time and searching efficiency of the parasitoid *Leptopilina clavipes* (Hartig). Accepted by *Journal of Animal Ecology*.

Kalbfleisch, J.D. & Prentice, R.L., 1980. *The statistical analysis of failure time data*. Wiley and Sons New York.

Krebs J.R., 1973. Behavioural aspects of predation. In: *Perspectives in Ethology* (ed. by P.P.G. Bateson & P.H. Klopfer): 73-111. Plenum Press, New York.

Lenteren, J.C. van, Nell, H.W. & Sevenster-van der Lelie, L.A., 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. *Journal of Applied Ecology* 89: 442-454.

Murdoch, W.W. & Oaten, A, 1975. Predation and population stability. *Advances in Ecological Research* 6: 2-132.

Noldus, L.P.J.J., 1991. The Observer: a software system for collection and analysis of observational data. *Behaviour Research Methods, Instruments & Computers* 23: 415-429.

Roermund, H.J.W. van & van Lenteren, J.C., 1992. The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) XXXIV. Life-history parameters of the greenhouse whitefly, *Trialeurodes vaporariorum* as a function of host plant and temperature. *Wageningen Agricultural University Papers* 92.3: 1-102.

Roermund, H.J.W. van, Hemerik, L & van Lenteren, J.C., in press. The influence of intra-patch experiences and temperature on the time allocation of the parasitoid *Encarsia formosa* foraging for whitefly on tomato leaflets. Submitted to *Ecological Entomology*.

Roermund, H.J.W. van & van Lenteren, J.C., in prep. Simulation of the foraging behaviour of the parasitoid *Encarsia formosa* during a single visit of a leaf.

Vianen, A. van & van Lenteren, J.C., 1986. The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). XV. Oogenesis and oviposition by *Encarsia formosa*. *Journal of Applied Entomology* 102: 130-139.

Vianen, A. van & van der Veire, M., 1988. Honeydew of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), as a contact kairomone for its parasite *Encarsia formosa* Gahan. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* 53/3a: 949-954.

Waage, J.K., 1979. Foraging for patchily distributed hosts by the parasitoid *Nemeritis canescens*. *Journal of Animal Ecology* 48, 353-371.

FORAGING BEHAVIOUR OF *ENCARSIA FORMOSA* ON *GERBERA* AND TOMATO LEAVES: A COMPARISON

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Key words:
Encarsia formosa, *Trialeurodes vaporariorum*, ornamental, vegetable, foraging behaviour

Summary
Foraging behaviour of *Encarsia formosa* was observed and compared on two different host plants, *Gerbera* and tomato. On both plants the females accepted 40% of the hosts, greenhouse whitefly, for egg laying. The time the wasps spent until they found their first host was also the same, about 4.5 minutes. The handling time of the host was slightly shorter on *Gerbera*, due to a shorter period spent on ovipositing. Foraging behaviour of *E. formosa* on *Gerbera* and tomato is very similar.

INTRODUCTION

Biological control of the greenhouse whitefly, *Trialeurodes vaporariorum* by the parasitoid *Encarsia formosa* is achieved in many vegetable crops under glass in the Netherlands. The parasitoid has proven its success, also from a commercial point of view. Up to 90 % of the Dutch tomato and sweet pepper acreage is under biological control (Van Lenteren & Woets, 1988). In ornamentals biological pest control is desired for the same reason as in vegetables and research has started with *Gerbera* as first crop (Sütterlin et al., 1992). The searching and foraging behaviour of the parasitoid are important parameters to know for a new host plant when one wants to examine the potential of *E. formosa* as a biological control agent. In previous work of our group the walking speed of the wasps on leaves was measured on different *Gerbera* cultivars and compared to the walking speed on tomato (Sütterlin et al., 1992). While the cultivars were considerably different in hairiness related to density as well as hair shape, we hardly found differences in walking speed. Also the walking speed was not different on *Gerbera* compared to tomato and the percentage of time searching on the leaf (walking activity) was the same on both host plants (Van Roermund & van Lenteren, in prep., Sütterlin & van Lenteren, in press and Sütterlin et al., in press). However, acceptance of hosts for egg laying was found to be up to 70 % on tomato (Van Roermund & van Lenteren, in prep.) and only about 40 % in pilot studies on *Gerbera* (Sütterlin et al., in press). This led to the question whether differences in acceptance by *E. formosa* were consistent on both host plants, and whether the encounter rates with and the handling of the host were the same on *Gerbera* and tomato?

MATERIAL AND METHODS

First experiment.

Host plants and whitefly hosts. On *Gerbera* plants, cultivar Tennessee, and tomato plants, cultivar Moneymaker, whitefly adults had the opportunity to lay eggs during 24 hours. The plants and the eggs laid developed in a different, whitefly- and *E. formosa* free glasshouse compartment at 20-22°C and 60 % relative humidity. After approximatly three weeks L_3/L_4 stages of the whitefly were present in a density of more than 50 larvae per leaf.

Parasitoids. E. formosa originated from Koppert Biological Systems. Only newly emerged females were used in the experiments (less than 16 hours old, females had the opportunity to feed on honey).

Experimental procedure. In a climate room (temperature 23-25°C, 70 % relative humidity) one heavily infested leaf was put in a glass vial into the pot of a clean plant, which was *Gerbera* or tomato, surrounded by a semicircle of clean plants of the same species and cultivar. A single *E. formosa* female was released next to a host on the leaf and observed. The behaviour was registered on a microcomputer with the software 'The Observer' (Noldus, 1991). As soon as the first host encountered was left by the wasp, we removed the female and took away the host carefully to examine it later for parasitoid egg(s).

Second experiment.

Host plants and whiteflies. Sixteen L_3/L_4 larvae were glued in a circle (3 cm diameter) with a tiny droplet honey on clean leaves of both host plant species. We used new leaf material every day.

Experimental procedure. A parasitoid female was released in the center of the circle just before the observation started. Detailed observations of the foraging behaviour were made and registered as described above. *E. formosa* that left the circle without encountering one of the hosts, parasitoids that flew away or did not move during one hour were not considered in the analysis.

RESULTS

Acceptance. In the first experiment we used 87 females on *Gerbera* plants and 79 on tomato. *E. formosa* accepted the same percentage of hosts for egg laying on both plants (40 % and 38 % respectively) (figure 1).

In the second experiment we tested 146 wasps on *Gerbera* leaves and 125 on tomato leaves. On each plant 51 females had an encounter with a host. The percentages of acceptance of hosts were almost identical to the first experiment, 39.22 % for *Gerbera* and 37.26 % for tomato plants.

The time spend on the leaf until the first encounter was 254.1 +/- 187.6 seconds on *Gerbera* and 270.3 +/- 248.7 seconds on tomato respectively.

Handling time. After encountering a host, a female can accept that host for egg laying or host feeding, or it can reject the host after antennal or ovipositorial contact. All behavioural elements have been observed on both host plants. Host feeding hardly occurred, only once on *Gerbera* and twice on tomato and the data

were therefore not taken into account in the analysis. Rejection with the antennae took a much shorter time than with the ovipositor on both host plants and rejection times were similar (figures 2 and 3). The time to lay an egg was significantly shorter on '*Gerbera* hosts' than on 'tomato hosts' (Wilcoxon test, p = 0.0253) (figures 2 and 3).

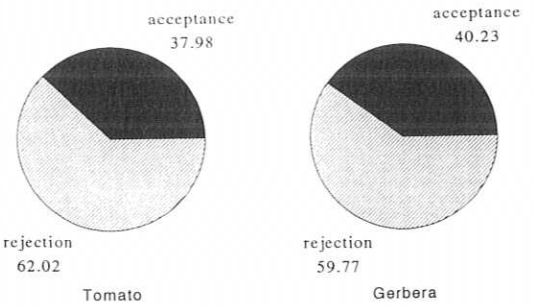

Figure 1. The acceptance of first encountered whitefly hosts for egg laying by *Encarsia formosa* females on tomato and *Gerbera* plants.

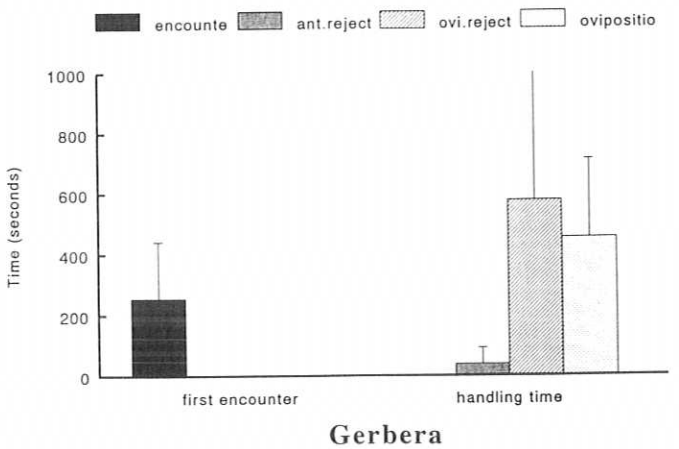

Figure 2. The time until the first encounter and the handling time of the host by *Encarsia formosa* females on *Gerbera* leaves (error bar = s.d.).

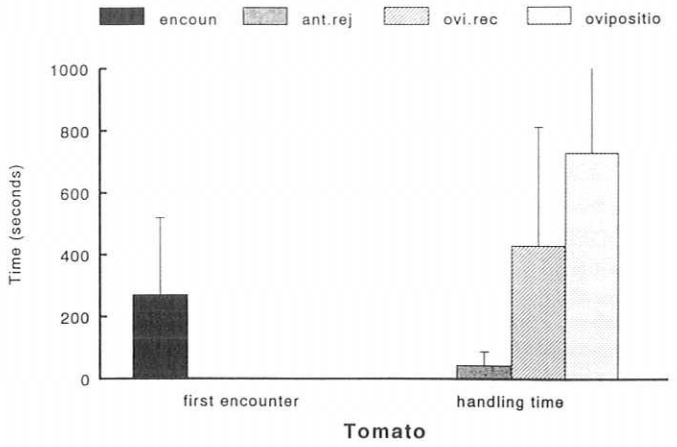

Figure 3. The time until the first encounter and the handling time of a host by *Encarsia formosa* females on tomato leaves (error bar = s.d.).

CONCLUSION AND DISCUSSION

* Host acceptance for egg laying at the first encounter is the same on *Gerbera* and tomato, 40 %. The acceptance on tomato plants found here is much lower than found by Van Roermund & van Lenteren (in prep.). However, experiments have been carried out under the same circumstances and we have no explanation for this difference.
* The time spent by wasps until the first encounter is the same on tomato and *Gerbera* (about 4.5 minutes).
* For host handling we found:
  - the same time is needed for host rejection (antennal rejection about 40 seconds and ovipositorial rejection about 8 minutes)
  - a significant difference between the time needed for oviposition on tomato and *Gerbera*; it is shorter on *Gerbera*. The time needed for oviposition on tomato hosts is exceptionally long when compared to literature data. Van Roermund & van Lenteren (in prep.) for example found a value of 362.0 seconds on tomato. Again, we have no explanation for this difference. However, in a sensitivity analysis of a simulation model of the foraging behaviour of *E. formosa* on tomato the handling time of the host is described as a parameter not important concerning the performance of the parasitoid in a 'natural environment' with a low host density (Van Roermund & van Lenteren, in prep. (b)).

On both host plants examined in this study we found a similar foraging behaviour. This, together with developmental and population dynamical data for host and

parasitoid (Van Roermund & van Lenteren, 1992 and Sütterlin & van Lenteren, in prep.), makes us believe that biological control of whiteflies by *E. formosa* is feasible in the ornamental *Gerbera*.

Acknowledgements
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REFERENCES

LENTEREN VAN, J.C. & J. WOETS, 1988 - Biological and integrated pest control in greenhouses. Annu. Rev. Entomology, 33, 239-269.

NOLDUS, L.P.J.J., 1991 - The Observer: A software system for collection and analysis of observational data. Behavior Research Methods, Instruments & Computers, 23 (3), 415-429.

ROERMUND, H.J.W. VAN & VAN LENTEREN, J.C., in prep. - Direct observations on the foraging behaviour of the whitefly parasitoid *E. formosa* on a leaf. Submitted to Environmental Entomology.

ROERMUND, H.J.W. VAN & VAN LENTEREN, J.C., in prep. - A population dynamicmodel of *Trialeurodes vaporariorum* (Westwood) and *Encarsia formosa* on a leaf. Submitted to Environmental Entomology.

ROERMUND, H.J.W. VAN & VAN LENTEREN, J.C., 1992 - The parasite - host relationship between .... XXXIV. Life-history parameters of the greenhouse whitefly, *Trialeurodes vaporariorum*, as a function of host plant and temperature. Wageningen Agricultural University Papers 92-3, 147 pp.

SÜTTERLIN, S. & VAN LENTEREN, J.C., in prep. - Developmental time of the greenhouse whitefly, *Trialeurodes vaporariorum*, on the ornamental *Gerbera jamesonii*.

SÜTTERLIN, S. & VAN LENTEREN, J.C., in press - Biological control of the greenhouse whitefly on the ornamental *Gerbera jamesonii*: How does *Encarsia formosa* behave in a patch? Part II. Bull. IOBC/WPRS (Workshop, April 25-29 1993, Asilomar, California).

SÜTTERLIN, S., VAN LENTEREN, J.C. & FRANSEN, J.J., 1990 - Dispersal of the greenhouse whitefly, *Trialeurodes vaporariorum* (Wesrwood), on the rosette plant *Gerbera*. Bull. IOBC/WPRS XIII/5, 205-208.

SÜTTERLIN, S., VAN RIJSOORT, J. & VAN LENTEREN, J.C., 1992 - Does the leaf surface of a *Gerbera* plant influence the searching behaviour of the parasitic wasp *Encarsia formosa*? Proc.Exper. & Appl.Entomol., N.E.V. Amsterdam, Vol. 3, 19-24.

SÜTTERLIN, S., VAN LEEST, A. & VAN LENTEREN, J.C., IN PRESS - Biological control of the greenhouse whitefly on the ornamental *Gerbera jamesonii*: How does *Encarsia formosa* behave in a patch? Bull. IOBC/WPRS (Workshop, September 8-11, Cambridge).

FINDING FLORAL NECTAR AND HONEYDEW IN *COTESIA RUBECULA*: RANDOM OR DIRECTED?

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Summary

The role of sugars in increasing longevity was investigated for *Cotesia rubecula*. Provision of sugar water prolonged the average life span of both sexes by a factor 9 and 14 respectively.

The response of parasitoids to flowers (floral nectar) and aphid infested leaves (honeydew) was tested in a y-tube olfactometer. Irrespective of their state of hunger, parasitoids were attracted to flower odours. Parasitoids did not respond to aphid infested leaf material.

INTRODUCTION

The importance of finding food for survival has been described for many parasitoid species (Zoebelein, 1955; Leius, 1967; Jervis and Kidd, 1986). However, the question of how parasitoids find their food sources under natural conditions has only been investigated to a limited extent.

Since *Cotesia rubecula* feeds on nectar, while parasitizing herbivorous *Pieris* spp., the process of food foraging is dissociated from host foraging. In the field, nectar-feeding parasitoids have various sugar sources available. Besides floral nectar, they can use honeydew, and extra-floral nectar for feeding (Leius, 1960). In our experiments, we first determined the effect of feeding on parasitoid survival. Subsequently we investigated the innate response of starved and satiated parasitoids to flowers (floral nectar) and aphid infested leaves (honeydew).

MATERIALS AND METHODS

Cotesia rubecula were reared as described by Wiskerke and Vet (1991). Satiated parasitoids were provided with sugar water (70%) and water. Starved parasitoids were given water only.

Survival experiment

To determine the survival of *C. rubecula* in the presence and absence of sugar water, one hundred individuals of either sex were divided upon emergence over ten plastic containers (12 x 12 x 8 cm) and kept at 25°C and a 16L:8D photocycle. Daily, one half of the containers were provided with both water and sugar water (70%), presented on separate cotton-wool plugs (satiated). The other half were given water only (starved). Daily counts were made of the number of surviving individuals per treatment.

Olfactometer experiment

One-two day old females were used in the olfactometer experiments.

Y-tube olfactometer. The olfactometer used was comparable to the one described by Steinberg et al., (1992). Various odour sources were tested in the y-tube olfactometer for their attractiveness to starved and/or satiated parasitoids.

Flowers and leaf material used in the Y-tube olfactometer were collected from the field. Flowers and leaf material were selected from plants free of any herbivore damage.

Ground-elder (*Aegopodium podagraria* L.; Umbelliferae) was chosen since flowers from the family of the Umbelliferae are known to be frequently visited by various parasitoid species (Kevan, 1973). Their exposed nectaries provide accessible nectar to nectar feeders with short mouth parts (Leius, 1960). A single umbel was used as an odour source in the choice experiments.

Rape seed (*Brassica napus* L.; Cruciferae) was chosen since it is known to be a host plant for *Pieris* spp. To match the biomass of one ground-elder umbel, ten rape seed flower heads were used as an odour source.

***Myzus persicae* on lettuce (*Lactuca sativa* L.) and rape seed leaves** were obtained from the greenhouse culture as described by Reinink et al., (1988). Infested leaves were covered with honeydew and contained 100-200 aphids of different instars, and their exuviae. Uninfested leaves were taken from aphid-free plants.

Test procedure

Parasitoid females were introduced in the central tube of the olfactometer, 1 cm from a start line. The observation started as soon as the parasitoid passed this start line. Walking upwind, the parasitoid could choose at the bifurcation between both olfactometer arms. The observation was counted as a choice when the individual passed the finish line in one of the arms for a period of 15 seconds. The small fraction (no more than 10% in any of the tests) of individuals that had not made a choice within 2 minutes were discarded. The connections between the odour source containers and the olfactometer arms were exchanged after every five parasitoids tested. Odour sources were renewed after every ten parasitoids tested. At the end of each day odour containers were cleansed with 70% ethanol.

RESULTS

Survival experiment. The availability of sugar water increased the life span of *C. rubecula* significantly for both sexes (Wilcoxon, $p<0.005$) (fig 1). Starved parasitoids lived an average of only 1.6 days (females) and 2.2 days (males), while the average life span for fed parasitoids was 23.2 days (females) and 19.5 days (males). When sugar water was available, females lived longer than males (Wilcoxon, $p<0.05$).

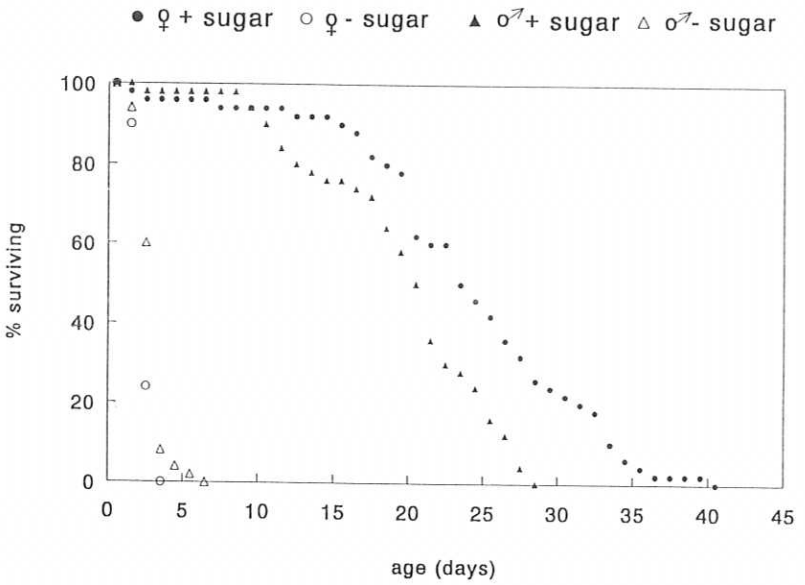

Fig. 1: Survival (in %) of *C. rubecula* in presence and absence of sugarwater at 25 C.

Olfactometer experiments. Starved as well as satiated parasitoids were attracted to flower odours. Both flowers tested (rape seed and ground elder) were chosen significantly more often than the corresponding undamaged leaf material (fig 2).

Starved parasitoids were not attracted to aphid infested rape seed- or lettuce leaves when tested against clean leaf material (fig 2).

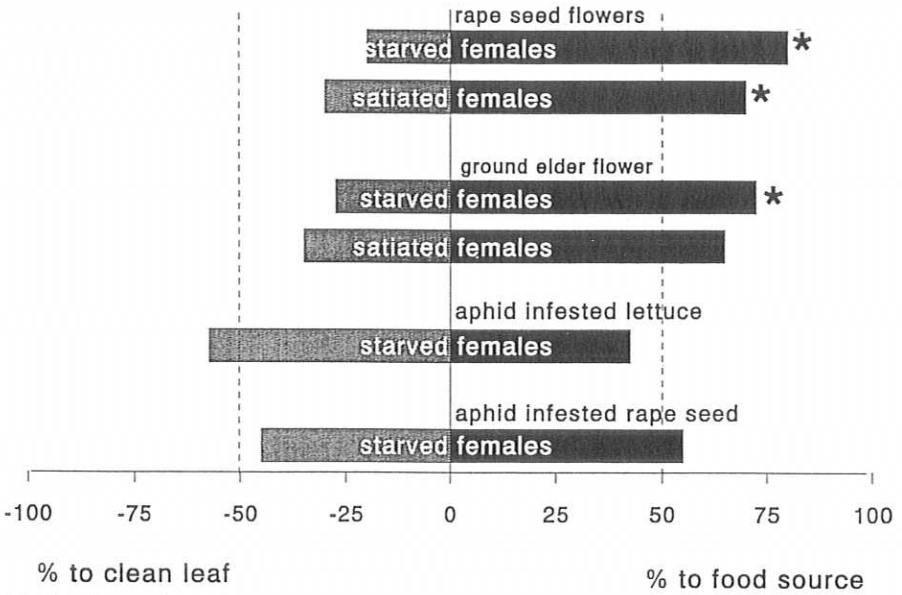

Fig. 2: Odour preferences of *C. rubecula* when given a choice in a y-tube olfactometer between various food sources and corresponding leaf material. N=40 for each comparison. Stars indicate a significant preference (test for binomial distribution; p=0.05).

DISCUSSION

Survival experiment: The importance of food for survival has been reported for many parasitoid species (Zoebelein, 1955; Jervis and Kidd, 1986). Our data for *C. rubecula* show that feeding increased the average life span of males and females by a factor nine and fourteen respectively. This underlines that the availability of food sources can be a crucial element in biological control.

For parasitoids like *C. rubecula*, that feed on food sources dissociated from the host sites, feeding represents a disruption of the host foraging process. When food is available in the direct vicinity of the host, this disruption will be minor. However, in the situation in which the host habitat does not provide food sources, food foraging can interfere considerably with parasitization efficiency. It is the latter situation that often applies to the agro-ecosystems in which natural enemies are released for biological control. Lack of suitable food could be an important cause of failure in biocontrol programmes (Clausen, 1956). Understanding mechanisms of food foraging in parasitoids can help us overcome these problems.

Olfactometer experiments: **Flower odours.** Both starved parasitoids and parasitoids satiated on sugar water were attracted by flower odours. This innate response will enable inexperienced parasitoids to locate floral nectar. The fact that *C. rubecula* responded both to flowers of a cruciferous and an umbelliferous plant, indicates that their flower odour preference is not restricted to the plant family on which these parasitoids find their hosts. Such "flower generalism" is adaptive when host infestation and nectar availability are not synchronised within a given plant species.

Aphid infested leaves. Besides floral nectar, parasitoids can feed on a variety of sugar sources (Kevan, 1973). Honeydew can be an important source of food and moisture, especially when flowering plants are scarce (Leius, 1960). Several papers report field observations of a wide variety of parasitoid species feeding on honeydew (Györfi, 1951; Zoebelein, 1955). Zoebelein (1955) showed that honeydew was indeed a suitable food source, increasing parasitoid longevity in all parasitoid species tested. Although honeydew will often be the most available and accessible sugar source under field conditions, we did not find attraction of starved *C. rubecula* to odours from honeydew, or aphid infested leaves. This lack of response, rather than reflecting a lack of interest, is probably a consequence of the fact that the parasitoids cannot perceive the presence of the food source. The latter is supported by the observation that starved *C. rubecula* readily assume feeding once honeydew has been contacted.

Unlike flowers, that advertise their nectar with notable scents and visuals in order to attract pollinators, there is usually little benefit to honeydew producers in attracting attention to their sugar excretion. To the contrary, since volatiles in honeydew can serve predators and parasitoids as kairomones leading to its producers, the latter are subject to a strong selection pressure to minimize honeydew detectability. This could explain the fact that even parasitoids of honeydew producing insects, to which honeydew could be a reliable indicator of host patches, do not seem to perceive honeydew volatiles (Sheehan and Shelton, 1989; Noldus and van Lenteren, 1990; Budenberg, 1990; Hågvar and Hofsvang, 1991).

Since *C. rubecula* neither responds to honeydew nor to volatiles of aphid infested leaves, finding honeydew is reduced to a random process. The chances of walking into honeydew are further reduced by the fact that *C. rubecula* mainly forages in flight and covers only a limited area after landing on the plant. For parasitoids like *C. rubecula* this means that honeydew will be of only limited value as a sugar source compared to the highly detectable floral nectar that the parasitoid can actively seek out.

REFERENCES

BARTLETT, B.R., 1964 - Patterns in the host feeding habits of the adult Hymenoptera. *Ann. Entomol. Soc. Am.* **57**,344-350.

BUDENBERG, W.J., 1990 - Honeydew as a contact kairomone for aphid parasitoids. *Entomol. Exp. Appl.* **55**,139-148.

CLAUSEN, C.P., 1956 - Biological control of insect pests in the Continental United States. *United States Dept. Agr. Tech. Bull.* 1139.

ELTON, C., 1966 - The pattern of animal communities. Methuen & Co. Ltd. London, 432 pp.

HÅGVAR, E.B. & T. HOFSVANG, 1991 - Aphid parasitoids (Hymenoptera, Aphidiidae): biology, host selection and use in biological control. *Biocontrol News and Information*, **12**,13-41.

JERVIS, M.A., & N.A.C. KIDD, 1986 - Host feeding strategies in Hymenopteran parasitoids. *Biol. Rev.* **61**,395-434.

KEVAN, G., 1973 - Parasitoid wasps as flower visitors in the Canadian high artic. *Anzeiger für Schädlingskunde, Pflanzen und Umweltschutz*, **46**,3-7.

LEIUS, K., 1960 - Attractiveness of different foods and flowers to the adults of some hymenopterous parasitoids. *Can Ent.* **92**,369-376.

LEWIS, W.J. & K. TAKASU, 1990 - Use of learned odours by a parasitic wasp in accordance with host and food needs. *Nature* **348**,635-636.

LUM, P.T.M., 1977 - Effect of glucose on autogenous reproduction of *Bracon hebetor* Say. *J. Georg. Entomol. Soc.* **12**,150-153.

NOLDUS, L.P.J.J. & J.C. VAN LENTEREN, 1990 - Host aggregation and parasitoid behaviour: biological control in a closed system. In: M. Mackauer, L.E. Ehler & J. Roland (eds) Critical issues in biological control. 292-262.

REININK K., F.L. DIELEMAN & R. GROENWOLD, 1988 - Selection of lines of lettuce (*Lactusa sativa* L.) with a high level of partial resistance to *Myzus persicae* (Sulzer). *Euphytica* **37**,241-245.

SHEEHAN, W. & A.M. SHELTON, 1989 - Parasitoid response to concentration of herbivore food plant: finding and leaving plants. *Ecology* **70**,993-998.

STEINBERG, S., M. DICKE, L.E.M. VET & R. WANNINGEN, 1992 - Response of the braconid parasitoid *Cotesia (=Apanteles) glomerata* to volatile infochemicals: effects of bioassay set-up, parasitoid age and experience and barometric flux. *Entomol. exp. appl.* **63**,163-175.

WISKERKE, J.S.C. & L.E.M. VET, 1991 - Comparison of two *Cotesia* species foraging for solitarily and gregariously feeding *Pieris* host species. *Proc. Exper. & Appl. Entomol., N.E.V.* **2**,190-196.

ZOEBELEIN, G., 1955 - Der Honigtau als Nahrung der Insekten, Teil I. *Z. Ang. Ent.* **38**,369-416.

RECRUITMENT AND FLIGHT ACTIVITY OF *MELIPONA FAVOSA*, FORAGING ON AN ARTIFICIAL FOOD SOURCE

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Keywords: *Melipona*, recruitment, foraging, flight activity.

Summary

This is our first study on the communication about food resources, and the recruitment of foragers in stingless bees (Apidae, Meliponinae). Experiments were undertaken with *Melipona favosa* under semi natural and experimental conditions in a large tropical greenhouse. A clear effect of recruitment was observed when individually marked foragers of *M. favosa* were trained on an artificial food source. A difference with natural situations was found in the intensity of nectar foraging of individual bees during the day. When the supply and the concentration of nectar were kept constant, no afternoon peaks in nectar foraging were found, as observed in nature.

INTRODUCTION

Little is known about the communication for food sources in the tropical stingless bees (Apidae, Meliponinae). Lindauer and Kerr (1958) found that in the genera *Melipona and Trigona* different kinds of communication exist. According to Esch and Kerr (1965) the most primitive communication can be found in *Trigona silvestrii*. In this species the foragers are only able to alert their nest mates to fly to an artificial nectar source if a perfume is added to the food. Esch and Kerr also observed the production of a weak sound when returning foragers enter the nest.

Other primitive types of communication were found in several small species, such as *Trigona jaty, T. muelleri, T. araujoi, and T. droryana*. Returning foragers of these species perform exited unstructured movements and produce a high-pitched buzz, consisting of long and short components, to recruit nestmates (Kerr & Blum 1981, Roubik 1989). Esch and Kerr (1965) found that these sounds were not correlated with the distance to the feeder. Played-back recordings of the sound stimulated the bees to move to the nest entrance but not to depart for foraging. Frequent interactions with the forager enables nestmates to detect food-odour. Now and then the forager stops to pass around small drops of nectar. With this information the recruits leave the nest and search for similar odours and tastes.

Foragers of *Trigona spinipes, T. hyalinata, T. trinidadensis, T. recursa, Oxytrigona tataira, Scaptotrigona postica, Cephalotrigona capitata, and Geotrigona mombuca* give additional information about the location of a food source (Kerr & Blum 1981, Roubik 1989). Scout bees mark the area of a food source with mandibular pheromones. While returning towards the nest a scentpath is produced by rubbing the mandibles on grasses and rocks every few meters. The scout then guides nestmates along this scent trail to the food (Lindauer & Kerr 1958).

Two species of *Melipona* (*M. seminigra and M. quadrifasciata*) were found to communicate about direction and distance of a food source without using chemical trails or leading guides. As in *Trigona,* returning foragers perform similar zig-zag runs, pass around food samples, and produce buzzing-sounds upon return in the nest. The time between two sound bouts is constant for all distances, but the length of each single bout is strongly correlated with the distance of the feeding place (Esch & Kerr 1965, Esch 1967).

Directional information is also given by the forager. Recruits usually run to the nest exit to follow a scout on a subsequent flight. The first part of this flight is marked by several erratic zig-zag flights in the general direction of the food source. The recruits first follow the forager a few times for about 10-30 meters and then return to the nest. After several such repetitions, some recruits take off and make their own way to the food site (Esch & Kerr 1965, Esch 1967).

The behaviour of returning foragers of *Melipona favosa* in the nest has been described by Sommeijer et al. (1983). Bees returning to the nest with pollen behaved differently from those returning with nectar. Nectar foragers perform the same behaviour as earlier described for other Trigona and Melipona-species. Although Sommeijer did not mention any sound production, it is likely that this also plays an important role in food communication in *M. favosa.* About 1.5 minutes after entering the nest the forager proceeds to the food storage pots to regurgitate the main nectar load. Returning pollen collectors run around with comparable excited movements, but instead of offering food they beg for it. On average 1 to 2 minutes after entering the nest the bee will unload the pollen in a storage pot, and leave the nest for another foraging flight.

In stingless bees different types of resources are being gathered at different times of the day. Peak pollen collection occurs in the early morning and peak nectar collection principally occurs in the afternoon (Sommeijer et al. 1983). Roubik among others, proposed that meteorological factors might have an obvious relation to foraging activity. Ambient temperature, insolation of flowers and humidity may affect nectar secretion and concentration (Roubik, 1989). A relationship between nectar quality and foraging activity was indicated by Roubik and Buchmann (1984). Foragers of *Melipona* collected higher viscosity nectar as the day progressed. At dawn bees normally start collecting pollen, and while pollen is depleted, nectar quality increases.

A study on pollen foraging by Sommeijer et al. (1983), revealed that 25% of the foragers in a colony of *M. favosa* collected both nectar and pollen at the same day, but doing so in separate flights. In that study it was not possible to observe a sequence in the collecting of nectar and pollen in individual foragers. Probably bees concentrate on collecting nectar when it is energetically the most profitable and abundant; when temperature is high and humidity low.

MATERIAL AND METHODS

All experiments were carried out in Burgers' Bush, a large tropical greenhouse (1.5 Ha) situated in Burgers' Zoo, Arnhem, the Netherlands. Single workers of *M. favosa* were trained on feeders, using a method described by Von Frisch (1965) for *Apis mellifera.* The feeder was positioned 4 meters in front of the nest, containing a 2M solution of honey in water. We worked with individually marked bees. In the experimental series all bees visiting the feeder were captured to obtain the number of visits that occur by chance. During the control series, trained bees were allowed to return to the nest in order to recrute nestmates to the feeder. All observation series had a duration of one day.

The time of the first arrival of each worker was recorded for each series, as well as the time of their subsequent visits during the rest of the day. For each forager the time spent on extranidal activities was recorded during foraging at the feeder. Intranidal observations were carried out for 26 half-hour periods, to record the behaviour of returning foragers in the nest.

RESULTS

Recruitment

The measurement of the time-interval between the first visits of two successive foragers revealed shorter intervals for the control series where the scout and the subsequent visitors were allowed to return to the nest.

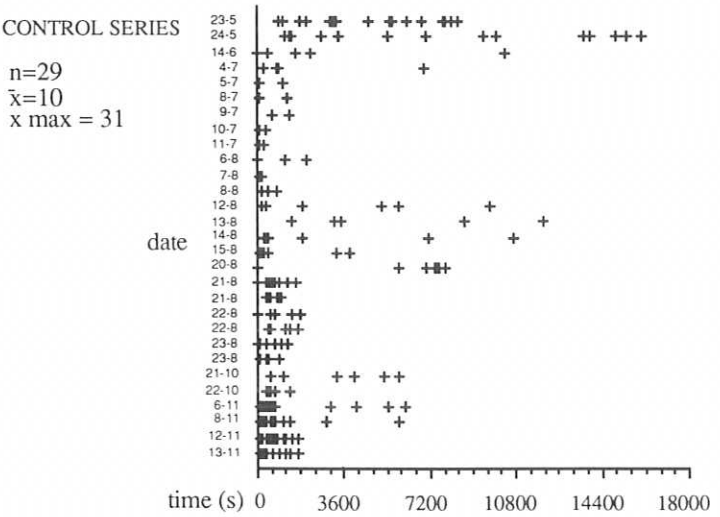

Fig. 1a. Arrival of foragers in control series.

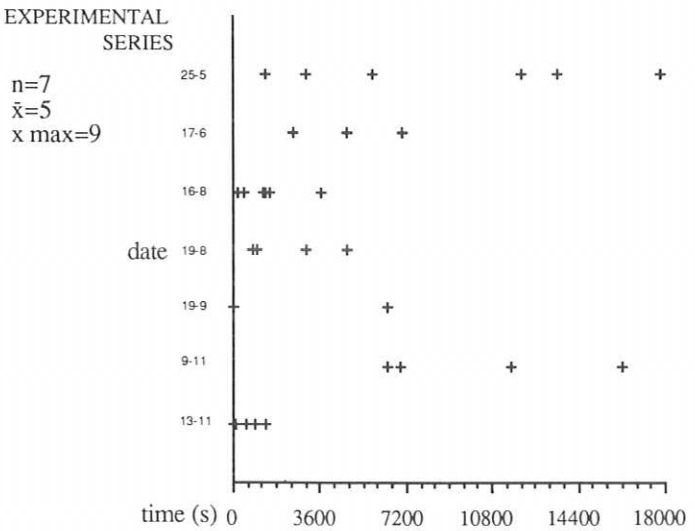

Fig. 1b. Arrival of foragers in experimental series.

In fig. 1, the time of the first arrival of successive foragers is represented by points at a time axis. Both the density of points near the vertical axis, and the number of points per observation day are higher in experimental series than in control series. This implies that the majority (84 %) of new foragers in the control series, arrive within half an hour after t =0. In the experimental series it clearly takes more time untill new foragers arrive at the feeder. The time interval between two successive foragers is bigger in experimental series. Only 35 % of the foragers arrive within the first half hour after t=0. In the control series the feeder is visited by twice as many foragers than in the experimntal series.

The two curves in fig. 2 respectively represent the probability by which specific intervals between two successive foragers occur in the control series and in the experimental series.

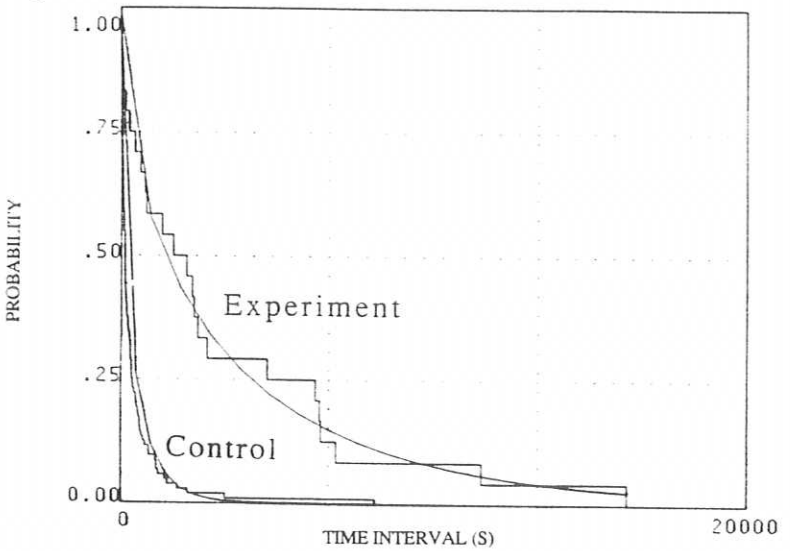

Fig. 2. Probability-distribution of time intervals in control and experimental series.

Flight activity

The flight activity of individual foragers, measured by the number of flights per hour, remained constant over the day. The average frequency was about 38 visits to the feeder per hour, per bee. Also the total number of visits at the feeder, by all foragers, remained constant over the day (fig. 3).

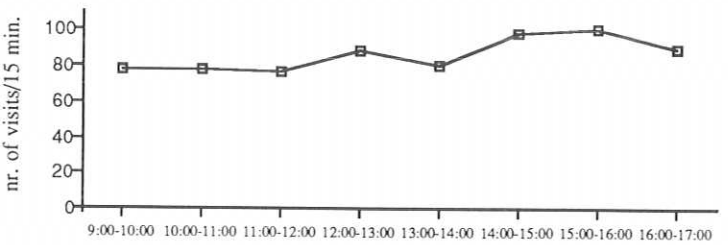

Fig. 3. Flight activity of M. favosa on the feeder over the day.

Intranidal behaviour of returning foragers

Foragers were found to behave in a clearly different way from their nestmates. Upon returning in the nest a forager often starts offering honey solution to one or more bees in the entrance tube. From here some returnees may depart again after only having exchanged a few food samples. Most of the time however (e.i. 69 %) the forager proceeds to the actual nest cavity. These foragers run around in a clearly excited zig-zag manner, performing several sharp turns in a semicircle (e.i. 180°). After each turn, food samples are offered to surrounding bees. Such trophallactic interactions may involve up to 4 bees at a time. Whithout having nestmates around, the returnee may start auto-grooming until a new bee approaches. On average one minute after entering the nest the forager departs for the feeder again.

Time budget of foragers

One foraging flight takes about 97 seconds. This includes flying to and from the feeder, food uptake, auto-grooming near the feeder and intranidal activities. The uptake of food takes approximately 20 seconds (20.56%). Subsequently the forager grooms at the feeder for about 8 seconds (8.11 %). Occasionally this is done on a plant within 2 meters distance of the feeder. Foragers were found to spend most of the time inside the nest; this was done on average for 63 seconds (64,37%, fig. 4).

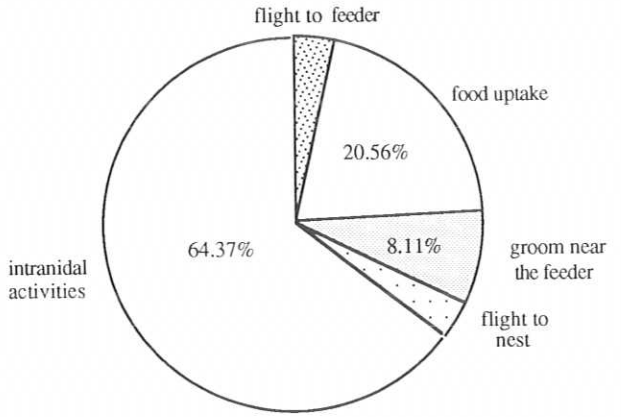

Fig. 4. Time budget of the different activities during foraging

DISCUSSION

Recruitment

When foragers are allowed to return to their nest, the time interval between the first visits of new visitors is short. On the other hand, when returning to the nest is prevented, this time interval is much longer. This indicates that the behaviour of the nestmates is influenced by the behaviour of returning foragers. From these results, including the observations on the interactions between returning foragers and nestmates, we can conclude that foragers of *Melipona favosa* recruite nestmates to a food source. This is clearly an example of communication. The details of resource communication in the nest may include several yet poorly understood variables. Intranidal observations, using individually marked foragers, might reveal new aspects of recruitment and communication in stingless bees, and of the evolution of communication in bees.

The occurrence of recruitment in stingless bees has not been established earlier in an experimental approach like ours. This method is especially fit for *Melipona* because of their relative small nests and small number of foragers.

Unloading nectar and pollen by returning foragers

The results of the observations on returning foragers concur with the observations described for *M. favosa* by Sommeijer et al. (1983) and for some *Melipona* and *Trigona* by Lindauer & Kerr (1958) and Esch & Kerr (1965). A very important difference with Sommeijers observations is that none of the returning foragers was ever seen depositing nectar in open nectar storage pots before leaving the nest. Returning pollen foragers however performed excactly the same behaviour as described by Sommeijer, including the unloading of their pollen loads in the storage pots.

Foraging and flight activity

Meteorological factors have an obvious relation to foraging activity (Roubik,1989). A clear effect of the meteorological factors on the foraging activity of bees in Burgers' Bush, was confirmed for *Tetragonisca angustula* (Kraaykamp, pers. comm.) and for *Melipona favosa* (De Bruijn et al., 1991).

In nature, stingless bees principally collect nectar in the afternoon, but under our semi natural circumstances the flight activity remained remarkably constant during the day. Keeping the amount and the concentration of the nectar in the feeder constant during the day apparently induced constant foraging. This might suggest that it are these variables that are responsible for major diurnal changes in nectar foraging known in natural situations.

The results of our study concur with the hypothesis (by Roubik, 1989) that meteorological factors do not influence the flight activity directly, but indirectly by differences in nectar secretion and dilution and pollen availability.

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REFERENCES

BRUIJN, L.L.M. de, M.J. VAN HERK and M.J. SOMMEIJER (1991) - Some observations on flight activity and foraging of the stingless bee *Melipona favosa* (Apidae, Meliponinae) in a large greenhouse. *Acta Horticulturae* **228**, 116-120.

ESCH, H. & W.E. KERR(1965) - Sound: An element common to communication of stingless bees and to dances of the honey bee. *Science* **149**, 320-321.

ESCH, H. (1967) - The evolution of bee language. *Scientific American* **216**(4), 97-104.

ESCH, H. (1967) - Die Bedeutung des Lauterzeugung für die Verständigung der stachellosen Bienen. *Z. Verg. Phys.* **56**, 199-220.

FRISCH, K. von (1965) - *Tanzsprache und Orientierung der Bienen*. Springer-Verlag, Berlin, Heidelberg, New York.

KERR,W.E. & BLUM (1981) - Communication of food source between workers of *Trigona spinipines*. *Rev. Brasil. Biol.* **41**(3), 619-623.

LINDAUER, M. & W.E. KERR (1958) - Gegenseitige Verstandigung bei den stachellosen Bienen. *Z. Verg. Phys.* **41**, 405-434.

ROUBIK D.W. and S.L.BUCHMANN (1984) - Nectar selection by *Melipona* and *Apis mellifera* and the ecology of nectar intake by bee colonies in a tropical forest. *Oecologia* **61**, 1-10.

ROUBIK, D.W. (1989) - *Ecology and natural history of tropical bees*. Cambridge Univ. Press, Cambridge, Mass.

SOMMEIJER, M.J., G.A. de ROOY, W. PUNT & L.L.M. de BRUIJN (1983) - A comparitive study of foraging behaviour and pollen resources of various stingless bees and honey bees in Trinidad, West-Indies. *Apidologie* **14**(3), 205-224.

DROSOPHILA PARASITOID SOLVES FORAGING PROBLEM THROUGH INFOCHEMICAL DETOUR: THE ROLE OF ADULT FLY PHEROMONE

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Key words: Leptopilina heterotoma, Drosophila simulans, infochemicals, kairomone

Summary

Parasitoids that forage for herbivorous hosts by using infochemicals may have a problem concerning the reliability and detectability of these stimuli. One solution to this problem is to learn to link highly detectable stimuli, e.g. stimuli from the host's food, to reliable but not very detectable stimuli. In this paper we report on another solution to the reliability-detectability problem. *Leptopilina heterotoma*, a parasitoid of *Drosophila* larvae, spies on the communication system of adult *Drosophila* flies to locate potential host sites. Naive parasitoids strongly respond to a volatile aggregation pheromone, *cis*-vaccenyl acetate (cVA), that is deposited in the oviposition site by recently mated female flies. Thus, *L. heterotoma* resorts to using highly detectable information from a host stage different from the one under attack.

INTRODUCTION

In a process of host-habitat location foraging parasitoids may use stimuli that are derived from their host or from the food of their host (Vinson, 1976; Nordlund et al., 1981). For an indication of host presence, accessibility and suitability, host-derived stimuli are the most reliable stimuli, but in general they are hard do detect from a distance. Stimuli from the food of the host are, on the contrary, easier to detect but are generally less reliable. Thus, foraging parasitoids are faced with a reliability-detectability problem for which they may have evolved different solutions (Vet & Dicke, 1992; Vet et al. 1991). One solution to the reliability-detectability problem is associative learning, a process through which parasitoids link detectable stimuli to reliable but hard-to-detect stimuli. Associative learning has proven to be an important solution for parasitoids in host and microhabitat location (see Vet & Groenewold, 1990; Vet & Dicke, 1992, for reviews). Another solution to the reliability-detectability problem is for parasitoids to resort to more conspicuous infochemicals from host stages different from the one under attack. Vet & Dicke (1992) called this solution the "infochemical detour". An example of the infochemical

detour is given by Noldus (1989), who mentions that *Trichogramma* egg parasitoids use a sex pheromone of the adult moth to locate a habitat where mating by the moths occurs and consequently host eggs may be found. In this paper we investigate the possibility that larval parasitoids of *Drosophila* employ an infochemical detour strategy by responding to a volatile aggregation pheromone of adult *Drosophila* flies.

The parasitoid in question is *Leptopilina heterotoma* (Thomson) (Hymenoptera: Eucoilidae), a polyphagous parasitoid that attacks larvae of many *Drosophila* species in a variety of substrates (Janssen et al., 1988). Ample knowledge is available on how associative learning aids *L. heterotoma* in microhabitat location (Vet, 1998; Vet & Groenewold, 1990; Papaj & Vet, 1990). In all these studies responses to odours from the food of the host were shown to be essential in host location an no evidence has ever been obtained that odours from the host itself (i.e. the larvae) play a role in microhabitat selection (Dicke et al., 1984; Vet, 1988). However, host-infested substrates were always prepared by adding host larvae to an uninfested substrate (Dicke et al., 1984; Vet, 1988).

According to research conducted by Bartelt et al. (1985) and Schaner et al. (1987), mature virgin *D. melanogaster* and *D. simulans* males produce an aggregation pheromone, which is attractive to flies of both sexes. For both *Drosophila* species, a major aggregation pheromone component was identified as (Z)-11-octadecenyl acetate (Z11-18:Ac), also known as *cis*-vaccenyl acetate (cVA). Virgin male flies, transfer cVA to virgin females during copulation. The mated female flies deposit the majority of the transferred cVA on the substrate within 6 hours after mating (Bartelt et al. 1985; Schaner et al. 1987). In this paper we investigate whether *L. heterotoma* "spies" on this *Drosophila* communication system.

MATERIALS AND METHODS

Flies. *Drosophila simulans* was reared on a medium of water, sugar, yeast and agar, in a weight ratio of 600:135:38:23. The flies were obtained from a laboratory culture, established from flies that were collected from a fruit orchard in the Netherlands. Pupae of *D. simulans* were washed and then individually placed in a small glass vial. The flies were maintained on water and honey in a 20 °C incubator. Approximately one week after emergence the flies were used.

Parasitoids. The parasitoids that were used in the experiments were 8 to 12 days old, naive *L. heterotoma* females. These females were obtained from a laboratory culture (on *D. simulans*), established from wasps that were collected from fermenting fruits in an apple-orchard in Wageningen in 1990. Females were maintained on water and honey in a 12.5 °C incubator. Several hours prior to an experiment, the parasitoids were transferred to 20 °C.

Windtunnel. In a windtunnel (100 * 60 * 40 cm), 2 odour sources were placed 10 cm apart on the glass floor. Female parasitoids were individually released from a glass vial, that was laid on the windtunnel floor 35 cm downwind from the 2 patches. A female was considered to have made a choice, when she had arrived on a patch and started probing the substrate with her ovipositor. Parasitoids that did not leave the vial within 5 minutes, that did not arrive on a patch within 10 minutes after leaving the vial or that flew away were recorded to be non-responsive. For each

experiment 50 females were tested.

Experimental set-up. For all treatments we used apple-yeast (AY) patches as a substrate. AY-substrate was prepared by mixing finely ground pulp of fresh apples (Golden Delicious) with fresh baker's yeast in a weight ratio of 15:1. These patches, with a diameter of 2 cm and a height of 0.5 cm, were placed on a circular piece of plastic with a diameter of 5 cm.

In a first set of experiments we investigated the response of *L. heterotoma* females toward patches on which different categories of *D. simulans* flies had been present prior to the experiment. Five types of patches were used:

1). 12 virgin *D. simulans* females and 12 virgin *D. simulans* males were placed on an AY-patch. Mating and oviposition occurred on this patch (fm);
2). 12 recently mated *D. simulans* females were placed on an AY-patch. Oviposition took place on this patch (f*);
3). 12 virgin *D. simulans* males were placed on an AY-patch (m);
4). 12 virgin *D. simulans* females were placed on an AY-patch (f);
5). a plain AY-patch (-).

According to Schaner et al. (1987) cVA is deposited on patches of categories 1, 2 and 3. The amount of cVA deposited by recently mated females (fm and f*) is however about 10 times larger than the amount deposited by virgin males (m) (Schaner et al. 1987). The flies were removed after a period of 6 hours, because a *D. simulans* female deposits the majority of the transferred cVA into the substrate within 6 hours after mating. Furthermore, we compared the response of females to 'natural' cVA with that of synthetic cVA. 4.5 µg of synthetic cVA (99% pure; Sigma Chemical Company), dissolved in 15 µl hexane, was added to an AY-patch. An individual *D. melanogaster* female emits approximately 0.30 µg of cVA within 6 hours after mating (Bartelt et al. 1985). As a control 15 µl of pure hexane was added to an AY-patch. Directly after preparation, the patches with synthetic cVA were used in the windtunnel.

In a second set of experiments we investigated how long AY-patches, on which adult *D. simulans* had mated and oviposited (fm), were attractive to naive *L. heterotoma* females. The AY-patches (fm) were stored in a 25 °C climate room. Under these conditions it takes 48 hours for the *D. simulans* larvae to reach a stage that can successfully be parasitized by *L. heterotoma*. On days 1, 2, and 3 (24, 48 and 72 hours after oviposition by *D. simulans*) female parasitoids were given a choice between an infested AY-patch (fm) and a plain AY-patch (-).

RESULTS

In Fig. 1 the results of the first set of experiments are presented. When 2 plain AY-patches (- vs. -) were offered in the windtunnel, approximately 25% of the parasitoids arrived on one of these patches. The response increased to about 80%, when a patch was offered on which *D. simulans* flies had previously mated and oviposited (fm). However, parasitoids did not distinguish between a patch on which *D. simulans* had mated and oviposited (fm) and a patch on which previously mated females had oviposited (f*). Females displayed a clear preference for patches on which mating and oviposition had occurred, when given a choice between these

patches and a patch on which either virgin males (m) or virgin females (f) had been present. Patches on which only virgin males had been present (m) were however attractive to *L. heterotoma* females when compared to a plain AY-patch (-). No significant difference was found when females had to choose between a patch on which only virgin *D. simulans* females had been present (f) and a plain AY-patch (-). Comparison of the patches with synthetic cVA (cVA) with the patches with 'natural' cVA (fm) showed that females had no preference for either of the two (Fig. 1). The last experiment in Fig. 1 shows that *L. heterotoma* females had a significant preference for an AY-patch with synthetic cVA (cVA) over an AY-patch with hexane (hex).

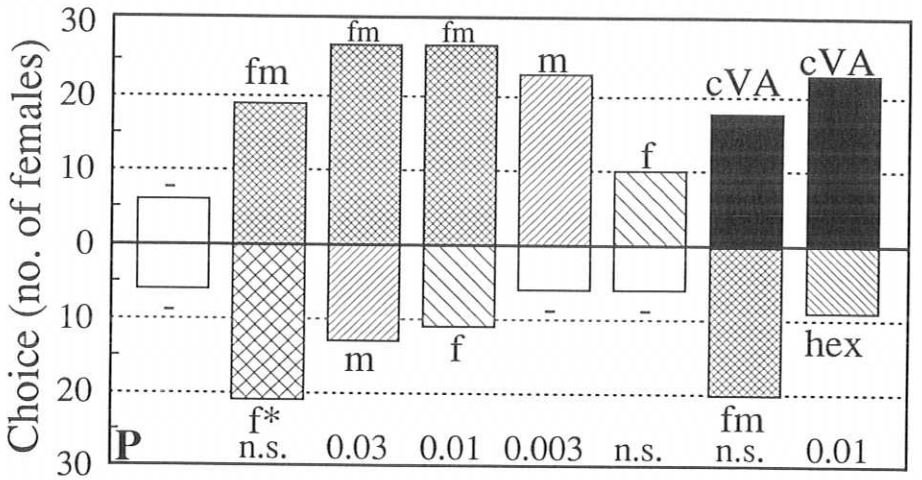

Fig. 1. *Response of naive L. heterotoma females toward patches on which different categories of D. simulans had been present (see experimental set-up), patches with synthetic cVA and patches to which hexane had been added. Choices were statistically analyzed by Chi-square test (n = 50 for each bar).*

In table 1 the results of the second experiment on days 1, 2 and 3 are presented. These show that the response of the females did not change significantly over a period of 3 days. Table 1 furthermore shows that on all 3 days females displayed a significant preference for infested AY-patches (fm).

Table 1. Response of *L. heterotoma* females toward infested AY-patches (fm) and plain AY-patches (-); 1, 2 and 3 days after deposition of natural cVA on the substrate.

	AY (fm)	AY (-)	Response	Chi-square
Day 1	17	6	72%	0.025
Day 2	24	1	74%	$4*10^{-6}$
Day 3	17	4	70%	$5*10^{-3}$

Day 1: n = 32; Day 2: n = 34; Day 3: n = 30.

DISCUSSION

Our results show that naive *L. heterotoma* females use cVA in host-habitat location. This volatile aggregation pheromone of several *Drosophila* species is a relatively well-detectable chemical. The reliability of cVA is higher than microhabitat volatiles; it is associated with oviposition activity of host flies as recently mated females deposit it in the substrate during oviposition (Bartelt et al., 1985; Schaner et al., 1987). As different stages of *Drosophila* (i.e. adult flies, eggs and larvae) often co-exist in the same microhabitat (Spieth, 1974; Shorrocks & Charlesworth, 1982) the presence of cVA very likely indicates the presence of larvae, the host stage that is parasitized by *L. heterotoma*. However, our results also show that patches, on which *D. simulans* had oviposited, are still attractive to naive *L. heterotoma* females 72 hours after oviposition occurred. By that time the patches contain the host stage that is preferably attacked by *L. heterotoma*, and therefore different stages of *Drosophila* do not necessarily need to co-exist in the same micro-habitat. Naive *L. heterotoma* females show relatively low responses to the odour of uninfested host substrates (Vet, 1988; Papaj & Vet, 1990). Substrates that are collected in the field induce relatively high responses from naive females in olfactometer tests (Janssen, unpubl. data; Vet, unpubl. data). It is, however, very likely that these field-collected substrates were contaminated with host pheromone, resulting in these high responses. If we assume that response to fly pheromone is a general phenomenon in *Drosophila* parasitoids, response to fly pheromone could offer an explanation for some of the results of the microhabitat choice experiments with a related species, *L. australis*, which parasitizes drosophilid flies in decaying petioles of giant hogweed (Van Alphen et al., 1991). Females of this species were more attracted by larvae-infested substrates than by uninfested substrates. Adult flies had been ovipositing on these infested substrates and therefore it is likely that pheromone was present. We believe that our findings shed a new and exciting light on niche differentiation and enemy-free space theory in these species. Responses to pheromones of adult flies may enable parasitoids to track their host species when these are invading novel substrates or when they are inhabiting less preferred substrates when food resources are limited.

Our findings have brought forward the fact that *L. heterotoma* has two solutions to the reliability-detectability problem when searching for larval hosts: the infochemical detour and associative learning. Naive females employ the infochemical detour by responding to the adult host's aggregation pheromone. After a successful oviposition, *L. heterotoma* uses associative learning during which the highly detectable and specific microhabitat volatiles are linked to an oviposition experience. Papaj & Vet (1990) demonstrated that associative learning increased their foraging efficiency in the field. However, these learned responses wane if continuation of reinforcement is absent, resulting in the parasitoids returning to their innate response pattern (Vet, 1988). Thus, the solution of associative learning is only present during a certain period after finding a suitable host.

For other results and a more detailed discussion about *L. heterotoma* employing the infochemical detour, we refer to Wiskerke et al. (1992).

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REFERENCES

ALPHEN, J.J.M. VAN, G. NORDLANDER & I. EIJS, 1991 - Host habitat finding and host selection of the *Drosophila* parasitoid *Leptopilina australis* (Hymenoptera, Eucoilidae), with a comparison of the niches of European *Leptopilina* species. *Oecologia* **87**, 324-329.

BARTELT, R.J., A.M. SCHANER & L.L. JACKSON, 1985 - *cis*-Vaccenyl acetate as an aggregation pheromone in *Drosophila melanogaster*. *J.Chem.Ecol.* **11**, 1147-1756.

DICKE, M., J.C. VAN LENTEREN, G.F.J. BOSKAMP & E. VAN DONGEN-VAN LEEUWEN, 1984 - Chemical stimuli in host-habitat location by *Leptopilina heterotoma* (Thomson) (Hymenoptera: Eucoilidae) a parasite of *Drosophila*. *J.Chem.Ecol.* **10**, 695-712.

JANSSEN, A., G. DRIESSEN, M. DE HAAN & N. ROODBOL, 1988 - The impact of parasitoids on natural populations of temperate woodland *Drosophila*. *Neth.J.Zool.* **38**, 61-73.

NOLDUS, L.P.J.J., 1989 - Semiochemicals, foraging behaviour and quality of entomophagous insects for biological control. *J.Appl.Entomol.* **108**, 425-451.

NORDLUND, D.A., R.L. JONES & W.J. LEWIS (eds.), 1981 - *Semiochemicals, their role in pest control*. Wiley, New York.

PAPAJ, D.R. & L.E.M. VET, 1990 - Odour learning and foraging success in the parasitoid, *Leptopilina heterotoma*. *J.Chem.Ecol.* **16**, 3137-3150.

SCHANER, A.M., R.J. BARTELT & L.L. JACKSON, 1987 - (Z)-11-octadecenyl acetate, an aggregation pheromone in *Drosophila simulans*. *J.Chem.Ecol.* **13**, 1777-1786

SHORROCKS, B. & P. CHARLESWORTH, 1982 - A field study of the association between the stinkhorn *Phallus impudicus* pers. and the British fungal-breeding *Drosophila*. *Biol.J.Linn.Soc.* **17**, 307-318.

SPIETH, H.T., 1974 - Courtship behaviour in *Drosophila*. *Ann.Rev.Entomol.* **19**, 385-405.

VET, L.E.M., 1988 - The influence of learning on habitat location and acceptance by parasitoids. *Colloq.INRA* **48**, 29-34.

VET, L.E.M. & M. DICKE, 1992 - Ecology of infochemical use by natural enemies in a tritrophic context. *Ann.Rev.Entomol.* **37**, 141-172.

VET, L.E.M. & A.W. GROENEWOLD, 1990 - Semiochemicals and learning in parasitoids. *J.Chem.Ecol.* **16**, 3119-3135.

VET, L.E.M., F.L. WÄCKERS & M. DICKE, 1991 - How to hunt for hiding hosts: the reliability-detectability problem in foraging parasitoids. *Neth.J.Zool.* **41**, 202-214.

VINSON, S.B., 1976 - Host selection by insect parasitoids. *Ann.Rev.Entom.* **21**, 109-134.

WISKERKE, J.S.C., M. DICKE & L.E.M. VET, 1992 - Larval parasitoid uses aggregation pheromone of adult hosts in foraging behaviour: a solution to the reliability-detectability problem. *Oecologia* (in press).

CLUTCH SIZE IN A LARVAL-PUPAL ENDOPARASITOID

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Key words: *Aphaereta minuta*, clutch size, reproductive strategy.

Summary

Clutch size decisions by *Aphaereta minuta*, a polyphagous, larval-pupal endoparasitoid were studied. This parasitoid attacks larvae of Diptera inhabiting ephemeral microhabitats. Females oviposit in young larval stages but the eventual size of the host pupa determines host food availability for competing offspring. We studied whether *A. minuta* females make clutch size decisions that benefit their fitness. Females vary their clutch size considerably and lay larger clutches in larvae of host species that produce larger pupae. They also lay larger clutches in bigger larvae than in smaller larvae of the same host species. We analyzed the relation between clutch size and fitness in different instars of the host *Delia antiqua*. Clutch size was artificially manipulated and the relation between clutch size and fitness was quantified using egg to adult survival, sex ratio and size of emerging adults. The calculated Lack clutch size (whereby fitness is maximized per clutch) increased with larval host stage. When host encounter rate is low, we expect clutch size to approach the Lack value. We found this to be the case.

INTRODUCTION

For an insect parasitoid the size of the host determines the amount of food available for the offspring. The capacity to allocate different numbers of offspring to hosts of different sizes, and so different quality, potentially enables polyphagous parasitoids to optimally exploit host resources. Indeed, as predicted by optimality models (Godfray, 1987), literature reports on a positive correlation between clutch size and host size (e.g. Takagi, 1986). Parasitoids that attack on-growing hosts have a difficult task to make optimal decisions on the allocation of the number of eggs to hosts, as the eventual size of the host has not yet been reached. The parasitoid *Aphaereta minuta* (Nees) (Hymenoptera: Braconidae) is a polyphagous larval-pupal endoparasitoid of differently sized fly species of which the larvae feed on ephemeral food sources. Young larvae are preferred for oviposition and the parasitoid eggs hatch during or just after the pupation of the host (Evans, 1933). Hence, at the moment of oviposition the female is faced with a problem of how to estimate the amount of resource that, after pupation of the host, is going to be available for its offspring. The foraging female parasitoid can encounter and parasitize larvae that greatly differ in age and size and

potential to grow further. How does she deal with such variation in resources and how is she to optimize the allocation of her offspring?

Optimality models have been developed to explain the evolution of clutch sizes and predict variability in this life-history parameter under various conditions such as limited resource (i.e. host) availability or limitation of time or eggs available in the foraging female wasps (Charnov & Skinner, 1984, 1985; Iwasa et al, 1984). Overall, when the relation between clutch size and fitness was quantified, observed clutch sizes were found to be lower than the calculated "Lack clutch size" (being the clutch size that leads to the greatest gain in parental fitness when maximizing the fitness gain per clutch is equivalent to maximizing lifetime fitness (Godfray et al, 1991)). The Lack clutch is only expected when animals lay a single clutch in their life or when opportunities to lay additional clutches are very rare. When eggs are limited, for example, females are selected to maximize their fitness gain per egg and so lay clutches of a single egg. When time available for oviposition is limited the animal is expected to maximize its overall rate of gain of fitness and clutch size will depend on the time between ovipositions, approaching the Lack clutch size only under long travel time conditions.

We investigate whether clutch size is influenced by the species, and size (or instar) of the host larva at the moment of oviposition.We manipulate clutch size and analyze how it affects parasitoid fitness in *D. antiqua* larvae of two different ages. Fitness curves are based on clutch size, survival to adult stage, sex ratio and fecundity of offspring. We also study the effect of host-encounter rate on clutch size decisions.

MATERIALS AND METHODS

Parasitoids

The culture of *A. minuta* originated from females that emerged from onion baits containing *D. antiqua* larvae collected near Wageningen, The Netherlands. *A. minuta* was maintained on first and second instar larvae of the onion fly *D. antiqua*.

Hosts

Larvae of different ages of *Delia antiqua* (Meigen)(Diptera: Anthomyiidae), *Phormia regina* (Meigen) (Diptera: Calliphoridae) and *Drosophila hydei* Sturt. (Diptera: Drosophilidae) were used as hosts. In case of *D. antiqua* we used first, second and third instar larvae. *D. antiqua* was reared on decaying onions at 23 °C, 70 % RH, *P. regina* on decaying meat at 23 °C and *D. hydei* on a yeast medium at 20 °C.

Experiments

Mated females were offered hosts in Petri dishes containing an agar layer with host rearing medium. Parasitoids were observed during oviposition and the number of quiverings of the last abdominal segments and the ovipositor sheath was counted (a direct determiner of clutch size). To analyze the correlation between host volume and clutch size, parasitized larvae were killed in hot water and length and width were measured. Others were incubated individually in a plastic cup (30 ml) containing a surplus of rearing medium. The number, sex ratio and size of their offspring was investigated. Clutch size was manipulated by interruption, superparasitization or low host-encounter rates (inter-oviposition time of 24 or 48 hours).

RESULTS

Clutch size in different host species and host ages.
In *D. hydei* and *P. regina* larvae, more eggs were deposited in older larvae and more eggs were laid in *P. regina* compared to *D. hydei* larvae of the same age (no data given). For *D. antiqua*, clutch size significantly increased with host larval age; 5.25, 8.50 and 10.57 eggs in first, second and third instar larvae, respectively. Clutch size increased with larval host volume in *D. antiqua* and *D. hydei* and more eggs were laid in *D. antiqua* compared to equally sized larvae of *D. hydei* (no data given).

Clutch size and fitness
Survival to adult stage, sex ratio and size (fecundity) of offspring were related to clutch size. Fitness is regarded as the number of eggs of offspring. For the calculation we used $F = c.s.e.sr$, where F = fitness, c = clutch size, s = survival to adult stage, e = number of eggs of offspring and sr = sex ratio (the fraction of daughters). The fitness curves are shown in Figure 1.

Effect of host-encounter rate
Clutch size in second instar *D. antiqua* larvae increased with inter-oviposition time (Fig. 2).

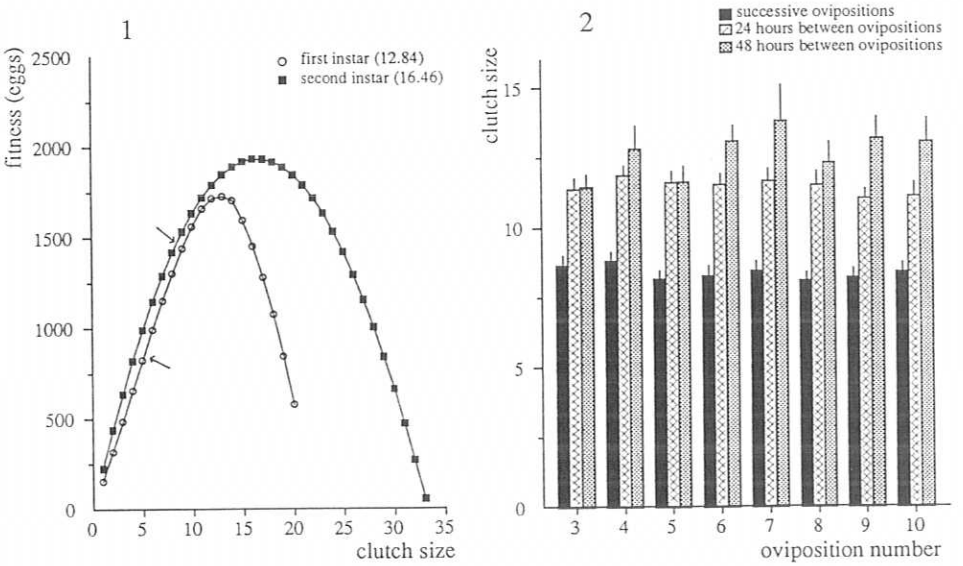

Fig. 1. *Fitness curves for parasitization of first and second instar* D. antiqua *larvae (Lack value in parentheses). Arrows indicate the observed clutch sizes.*

Fig. 2. *Clutch size for successive ovipositions of* A. minuta *in second instar* D. antiqua *larvae for different inter-oviposition times. Mean (bars) and SE (lines).*

DISCUSSION

Clutch size optimality models commonly predict that larger clutches should be placed on better-quality hosts (Godfray et al, 1991). For parasitoids this quality will be determined by host size. For several parasitoid species there is evidence of a positive relationship between clutch size and the size or instar of the host species (e.g. Hardy et al, 1992).

Within second instar *D. antiqua* larvae, clutch size also increased with host size, so host instar is not the only factor that determines clutch size. The results also showed that *A. minuta* is able to distinguish between host species with potentially different pupal sizes, depositing more eggs in equally sized larvae of species which develop into larger pupae. What we do not know however is how she distinguishes. The only means of contact the parasitoid has is through the ovipositor. The female has to assess both size and physiological age of the larvae. So far the cues involved can only be guessed.

A. minuta generally oviposits into young larvae. As the host larva is only at the beginning of its growth, the parasitoid has to estimate the expected pupal size because host size at parasitization is not a direct determiner of the amount of food available to the developing parasitoids. In addition she has to consider several factors that may reduce clutch survival. When parasitizing young larvae she has to account for a relatively high mortality of the host larvae. This relatively high mortality was found in the laboratory under optimal conditions (surplus of food, no predators etc.) and is expected to be much more intense under field conditions. Diptera host larvae feed on ephemeral substrates and food competition can be very intense due to the high number of fly eggs laid and the scramble type competition. Many young larvae never make it to the pupal stage. Furthermore, the longer period of exposure to natural enemies imposes an extra mortality risk for younger larvae.

The calculated Lack clutch size differs between larval stages. It is lower in younger larvae than in older larvae. We calculated the Lack clutch size using survival, sex ratio and size-related fecundity. In our calculations we considered longevity and other factors like behavioural characteristics to be constant. Behavioural characteristics, such as host selection, host discrimination and searching ability will also determine the reproductive success of a female under field conditions. The data presented here show that the observed clutch sizes lay below the calculated Lack clutch sizes. This is in accordance with earlier studies with parasitoids (e.g Waage & Ng, 1984; Hardy et al, 1992). This may be due to an overestimation of the Lack clutch size (Hardy et al, 1992). The correct calculation of the Lack clutch size depends on the knowledge of the relationship between adult size and realized fitness. However, perhaps it is more likely that fitness maximization per clutch (Lack) is, in fact, not functional. This will be the case when reproductive success is not limited by the opportunity to produce more than one clutch, but by other factors such as time or eggs. Whether *A. minuta* is time- or egg-limited remains unclear without field data. As expected, an increase in time between ovipositions is pushes the observed clutch towards the Lack value (Charnov and Skinner, 1984, 1985).

REFERENCES

CHARNOV, E.L. & S.W. SKINNER, 1984 - Evolution of host selection and clutch size in parasitoid wasps. *Flor.Entomol.* **67**, 5-21.

CHARNOV, E.L. & S.W. SKINNER, 1985 - Complementary approaches to the understanding of parasitoid oviposition decisions. *Env.Entomol.* **14**, 383-391.

EVANS, A.C., 1933 - Comparative observations on the morphology and biology of some hymenopterous parasites of carrion-infesting Diptera. *Bull.Entomol.Res.* **24**, 385-405.

GODFRAY, H.C.J., 1987 - The evolution of clutch size in invertebrates. *Oxford Surveys in Evolutionary Biology* (ed. by P.H. Harvey & L. Patridge), **4**, pp. 117-154, Oxford University Press, Oxford.

GODFRAY, H.C.J, L. PATRIDGE & P.H. HARVEY, 1991 - Clutch size. *Ann.Rev. Ecol.Syst.* **22**, 409-429.

HARDY, I.C.W., N.T. GRIFFITHS & H.C.J. GODFRAY, 1992 - Clutch size in a parasitoid wasp: a manipulation experiment. *J.Anim.Ecol.* **61**, 121-129.

IWASA, Y., Y SUZUKI & H. MATSUDA, 1984 - Theory of oviposition strategy of parasitoids. I. Effect of mortality and limited egg number. *Theor.Pop.Biol.*, **26**, 205-227.

TAKAGI, M., 1986 - The reproductive strategy of the gregarious parasitoid, *Pteromalus puparium* (Hymenoptera: Pteromalidae) 2. Host sizediscrimination and regulation of the number and sex ratio of progeny in a single host. *Oecologia* **70**, 321-325

'DIVISION OF LABOUR' IN HONEYBEES: A REVISION

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Key words: *Apis mellifera*; division of labour; individual differentiation; juvenile hormone; self-structuring; social organisation

Summary

Traditional studies concerning behaviour in honeybees almost entirely concentrates on 'age-dependent division of labour'. In this paper, recent work with respect to the influence of genotypic differences, the role of juvenile hormone and individual differentiation is discussed. It is concluded that it is not useful anymore to consider 'age-dependence' as the most important factor with respect to a workers' behaviour.

Introduction

'Age-dependent division of labour' has been the main issue in classical studies concerning honeybee behaviour. Recently, it has become evident that only very few behavioural switches do in fact relate to the age of the worker. The most important of these switches, from household worker to forager, is now regarded to be the result of a change in juvenile hormone (JH) titer. However, the development of the JH titer is not an autonomous process since it is very much influenced by the environmental situation. Furthermore, it became clear that there are many behavioural acts for which differentiation occurs which is not related to age. Although these differences are partly explained by genotypic differences, the proximate mechanisms leading to behavioural differentiation at the level of individuals still largely remain to be revealed. The aim of this paper is to summarise the most important aspects concerning behavioural differentiation in workers and to speculate about the proximate processes underlying this differentiation.

Age-related behaviour

Early papers on 'division of labour' (Rösch, 1925, 1930) suggest that each worker carries out the same innate sequence of age dependent activities. Later authors (Lindauer, 1952; Michener, 1974; Seeley, 1982; Kolmes, 1985) showed that age dependent behavioural programming is not as strict as previously assumed. Most workers perform a lot of different behavioural acts ('tasks') within one day of observation. Initially, no statistics were applied in order to discriminate between the age groups performing different behaviour. Applying statistics, Seeley & Kolmes (1991) found only two significant behavioural changes during a workers' life. Firstly, until they are four days old, workers mostly stay inside empty cells, a behaviour that has been interpreted as 'cell cleaning' by most of the authors (Seeley, 1982; Seeley and Kolmes, 1991). After this, they start to

move around in the hive and perform a great variety of behaviour in the centre of the nest (e.g. brood care, cell capping, queen attending). A second significant switch takes place when the workers are between 2 and 3 weeks old. At this stage, workers start to work in the periphery of the nest (pollen storage, nest defending), after which they end as a forager.

A lot of information has become available concerning these two behavioural switches. Behaviour of young workers was studied by van der Blom & van Oosterhout (in prep.). They found that, although young workers spend between 80 and 50 percent of the time in empty cells, most of the workers in empty cells are completely motionless in a characteristic 'upside down' position. Actual 'cleaning' (as described by Lindauer, 1952) involves turning around the body axis and treating the cell walls with the mandibles. It may therefore be concluded that young workers do not clean cells as frequently as previously assumed. There seems to be no justification for attributing such a specific role to the young workers ('nest cleaners') in the system of 'division of labour'. Staying in empty cells merely seems to be a kind of extension of the process of maturation (e.g. in order to blow up the wings, to stiffen their initially soft chitinous parts and to develop muscles).

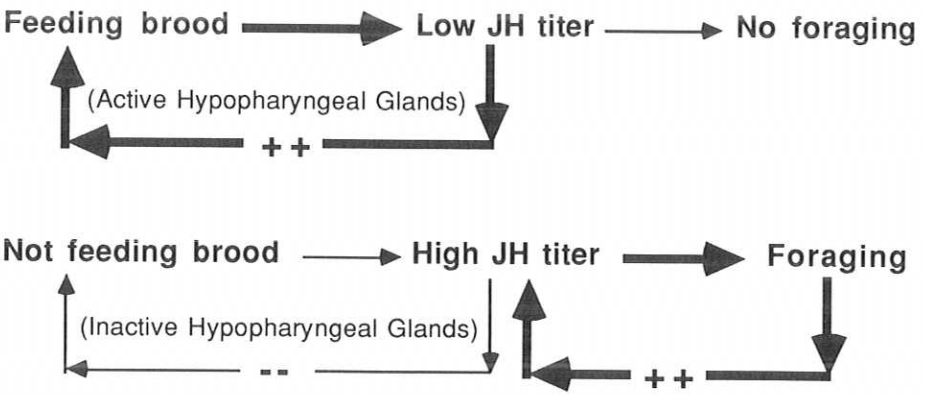

Figure 1. Physiological feedback in the individual regulation of 'household behaviour' or working in the periphery and foraging.

The age of becoming forager may vary considerably in response to changing conditions (Winston & Punnet, 1982; Winston & Fergusson, 1985, 1986; Fergusson & Winston, 1987; Kolmes, 1990). Recent studies concerning the role of juvenile hormone (JH) in the regulation of worker behaviour have revealed important aspects concerning this switch. 'Nurse bees', workers performing behaviour in the centre of the nest (e.g. brood care), have a low JH titer, whereas foragers (which may be of the same age) have a high JH titer. Idiosyncrasy among equally aged workers can be induced by injecting part of the worker group with JH (reviewed by Robinson, 1992). The JH titer does not develop independently (e.g. as a function of age), but is greatly influenced by the situation in the colony. Manipulations, as a result of which workers are forced to change

their behaviour, may cause either young workers to get a high JH titer, like normal old foragers, or old workers to get a low JH titer, like young hive bees. There is a connection between the activity of the hypopharyngeal glands and the JH level: a low JH titer seems to favour a high activity of the glands, whereas the hypopharyngeal glands are reduced once the JH titer rises (Imboden & Lüscher, 1975). The age at which the switch from 'household worker' towards forager takes place thus depends on the situation in which workers are. JH acts as a physiological amplifying mechanism once behavioural changes have started (e.g. once a worker for some reason loses contact with the brood). This is diagrammatically shown in Figure 1. The use of the terms 'Feeding -' or 'Not Feeding Brood' in this figure is a simplification, since interactions between workers, during which proteins may be exchanged by means of trophallaxis (Crailsheim, 1990), may have the same effect (Robinson, 1992).

Recently, Tofts & Franks (1992) explained the problems of a rigid age-dependent system of division of labour. Furthermore, they pointed out that age-related behaviour can occur without a causal link between the age of a worker and the task she performs. The switch as illustrated in Fig. 1 is a good example of this principle.

Self structuring

The switch from household worker to forager is an example of a change in behaviour which is regulated by a (in this case physiological) feedback mechanism. This principle is also known with respect to foraging behaviour of honeybee workers. If a worker arrives at a flower and finds a valuable amount of food there, she will quickly develop a preference for that specific type of flower (e.g. Menzel et al., 1973; Menzel & Erber, 1978). Preference is established by learning. Learning, in this case, is the mechanism through which feedback operates. In the last decade there has been an increasing number of publications which show that highly differentiated groups may develop in originally homogeneous populations as a result of feedback mechanisms (e.g. Hogeweg & Hesper, 1981; Deneubourgh et al., 1987; Plowright & Plowright, 1988). With respect to honeybee behaviour inside the hive, this aspect has not been given much attention until now. Calderone & Page (1991); Page & Mitchell (1991) and Page & Robinson (1991) do not include changes in individual thresholds as a result of feedback mechanisms (e.g. through learning) in their models of division of labour. They emphasise the influence of genotypically determined thresholds, which predominantly change as a function of age.

Genotypic variability

In the last decade, a great significance was attributed to genotypic influences in the performing of behaviour by workers (reviews by Calderone & Page, 1991; Page & Robinson, 1991 and Robinson, 1992). Although there is no doubt that genotypic differences between workers are important with respect to the kind of behaviour that is performed by individual workers, it is necessary to consider this in relation to the processes of behavioural development and the causal mechanism leading to specific behaviour. This is briefly summarised in Figure 2.

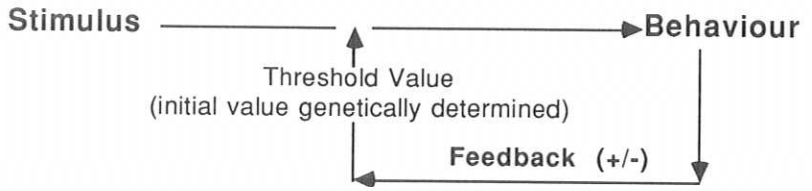

Figure 2. The individual threshold values for the onset of a behavioural act may initially be genotypically determined, but may be strongly influenced by feedback mechanisms.

First of all, the proper stimuli must be present to start a behaviour. These stimuli will usually not be homogeneously dispersed through the colony, so for individuals it is a matter of chance to meet a stimulus which exceeds the threshold level for a certain behaviour. The initial threshold level of these stimuli may most importantly be genotypically determined. Performance of a behaviour, however, may alter the threshold level with respect to this behaviour (e.g. through learning and the development of preferences). Changes in thresholds due to this kind of feedback may thus overrule the genotypic differences which were present originally. At colony level, this means that genetic differences will establish relative differences between groups of different genetic origin (because the initial chance that workers start the behaviour is bigger for the workers of one group than for workers of the other group), whereas at the level of individuals the genotypic differences do not imply deterministic proximate clues.

Individual differences between workers

In order to understand behavioural differentiation in honeybees, the behaviour has to be studied at the level of the individual. Kolmes (1989), van der Blom (1991; 1992; 1993) & van der Blom & van Oosterhout (in prep.) have shown that significant differentiation occurs with respect to some behavioural acts, whereas other behaviour seems to be performed at random by all workers. Significant idiosyncrasy has been found for 'staying in empty cells'; 'comb construction', 'allogrooming' and some behavioural acts which occur in queenless colonies: 'involvement in aggressive interactions' (both as 'aggressor' and as 'victim') and 'staying in empty cells'. The distributions of 'queen attending' and the number of times workers were allogroomed never deviated from random distributions. Also workers' involvement in 'visiting larvae in emergency queen cells' appears to be mostly random. Although until now only a limited number of behavioural acts have been studied, it is clear that a lot of differentiation in worker behaviour occurs for which we do not know the proximate cause. This differentiation has until now mostly been hidden, because it often takes place within groups of workers that were compared (e.g. within age groups).

Thusfar the studies were not designed to reveal proximate mechanisms behind individual differentiation. However, especially in the cases where no deviations from random distributions were found, these mechanisms may be very simple. E. g. a simple encounter with a queen may be sufficient as releasing stimulus to start queen attending

(van der Blom, 1992). Observed differences between workers may therefore be a consequence of spatial distribution of the workers. In the cases where significant idiosyncrasy was found, further research is required to study the process of differentiation (the ontogeny of the behaviour) of individual workers. It is likely that this process is at least in part controlled by feedback mechanisms which may amplify existing differences among workers (e.g. genotypic differences in initial threshold values), or may even generate significant differences within an initially homogeneous group.

Conclusions

It has now become obvious that a great deal of behavioural differentiation is not age related. An indirect relation with age can be found with respect to the transition from household worker to forager. Under normal circumstances, this is a rather definite switch, the timing of which depends on the situation in the colony. Many behavioural acts inside the colony, however, are not irreversibly initiated but are performed by temporal specialists. Similar to foraging workers, who develop a strong specialisation for certain flowers as a result of learning, it may be expected that also within the hive behavioural specialisation is largely the result of feedback mechanisms which amplify existing differences which may be genotypically based or random. This will have to be investigated experimentally in future research.

While studying the differentiation in worker behaviour, it is not useful to make the commonly used separation between 'tasks' and 'non tasks' (for definition see Seeley, 1982) with respect to an individual workers' development. Worker's activities are always composed of a mixture of both and the distinction can not easily be made (as became clear in our study with regard to 'cell cleaning'). This means that attention in future research should not be directed to 'regulation of division of labour', which is a functional concept from the point of view of colony needs, but to 'behavioural differentiation of individual workers', which reflects a more proximate ethological approach.

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References

van der Blom, J. 1991. Social regulation of egg-laying by queenless honey bee workers (*Apis mellifera* L.). *Behav. Ecol. Sociobiol. 29:* 341-346.

van der Blom, J. 1992. An analysis of queen attending by worker honey bees. *Ins. Soc. 39*: 237-249.

van der Blom, J. 1993. Individual differentiation in behaviour of honey bee workers (Apis mellifera L.). *Ins. Soc. 40*: in press.

Calderone, N.W. and R.E. Page, 1991. Evolutionary genetics of division of labor in colonies of the honey bee (*Apis mellifera*). *Am. Nat. 138*: 69-92.

Crailsheim, K., 1990. Protein synthesis in the honeybee (*Apis mellifera* L) and trophallactic distribution of jelly among imagos in laboratory experiments. *Zool. Jb. Physiol. 9:*, 303-312.

Deneubourgh, J.-L., S. Goss, J.M. Pasteels, D. Fresneau and J.-P. Lachaud, 1987. Self organisation mechanisms in ant societies (II): learning and foraging in division of labour. In: Pasteels, J.M. and Deneubourgh, J.-L. (Eds.): *From individual to collective behavior in social insects. Experientia (Suppl.) 54*: 177-196.

Fergusson, L.A. and M.L. Winston, 1987. The influence of wax deprivation on temporal polyethism in honey bee (*Apis mellifera* L.) colonies. *Can. J. Zool. 66*: 1997-2001.

Hogeweg, P. and B. Hesper, 1981. The ontogeny of the interaction structure in bumble bee colonies: a MIRROR model. *Behav. Ecol. Sociobiol. 12:* 271-283.

Imboden, H. & M. Lüscher, 1975. Allatektomie bei adulten Bienenarbeiterinnen (*Apis mellifera*). *Rev. Suisse Zool. 82*: 694-698.

Kolmes, S.A., 1985. A quantitative study of the division of labour among worker honey bees. *Z. Tierpsychol. 68*: 287-302.

Kolmes, S.A., 1989. Grooming specialists among worker honey bees, *Apis mellifera. Anim. Behav. 37*: 1048-1049.

Kolmes, S.A., 1990. Recent progress in the study of adaptive behavioural flexibility in honey bees. *Bee World 71*: 122-129.

Lenoir, A., 1987. Factors determining polyethism in social insects. In: J.M. Pasteels and J.-L. Deneubourgh (Eds.): *From individual to collective behavior in social insects. Experientia (Suppl.) 54*: 219-241.

Lindauer, M., 1952. Ein Beitrag zur Arbeitsteilung im Bienenstaat. *Z. verg. Physiol. 34*: 299-345.

Menzel, R., J. Erber, & T. Masuhr, 1973. Learning and memory in the honeybee. In: L.B. Browne (Ed.) *Experimental analysis of insect behaviour*. Springer, Heidelberg. pp: 195-217.

Menzel, R., J. Erber, 1978. Learning and memory in bees. *Sci. Am. 239*: 102-111.

Michener, C.D., 1974. *The social behaviour of the bees: a comparative study*. Belknap / Harvard Univ. Press, Camebridge MA.

Page R.E. and G.E. Robinson, 1991. The genetics of division of labour in honey bee colonies. *Adv. Insect Physiol. 23*: 117-169.

Page, R.E. and S.D. Mitchell, 1991. Self organisation and adaption in social insects. In: A. Fione, M. Forbes & L. Wessels (Eds.). *PSA 1990*. Phylosophy of Science Association, East Lansing Mich.

Plowright, R.C. and C.M.S. Plowrightt, 1988. Elitism in social insects: a positive feedback model. In: Jeanne, R.L. (Ed.). *Interindividual behavioral variability in social insects*. Westview Press, Boulder & London.

Robinson, G.E., 1992. Regulation of division of labor in insect societies. *Annu. Rev. Entomol. 37*: 637-665.

Rösch, G.A., 1925. Untersuchungen über die Arbeitsteilung im Bienenstaat, I. Teil: Die Tätigkeiten im normalen Bienenstaate und ihre Beziehung zum Alter der Arbeitsbienen. *Z. verg. Physiol. 2*: 571-631.

Rösch, G.A., 1930. Untersuchungen über die Arbeitsteilung im Bienenstaat, II. Teil: Die Tätigkeiten der Arbeitsbienen unter experimentell veränderten Bedingungen. *Z. verg. Physiol. 12*: 1-71.

Seeley, T.D., 1982. Adaptive significance of the age polyethism schedule in honey bee colonies. *Behav. Ecol. Sociobiol. 11*: 287-293.

Seeley, T.D. & S.A. Kolmes, 1991. Age polyethism for hive duties in honey bees -- illusion or reality? *Ethology 87*: 284-297.

Tofts, C. & N.R. Franks, 1992. Doing the right thing: ants, honeybees and naked mole-rats. *TREE 7*: 346-349.

Winston, M.L and E.N. Punnett, 1982. Factors determining temporal division of labor in honey bees. *Can. J. Zool. 60*: 2947-2952.

Winston, M.L. and L.A. Fergusson, 1985. The effect of worker loss on temporal caste structure in colonies of the honey bee (*Apis mellifera* L.). *Can. J. Zool. 63*: 777-780.

Winston, M.L. and L.A. Fergusson, 1986. The influence of the amount of eggs and larvae in honey bee (Apis mellifera L.) colonies on temporal division of labor. *J. Apic. Res. 25*: 238-241.

Winston, M.L., 1987. *The biology of the honey bee*. Cambridge (Massachusetts): Harvard University Press.

BIOSYNTHESIS OF HDLP AND APOLP-III BY THE FAT BODY OF THE MIGRATORY LOCUST

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Key words: locust, lipophorin, apolipophorin III

Summary

The fat body of the migratory locust synthesizes apolipophorin (apoLp) I, -II and -III. ApoLp-I (220 kDa) and apoLp-II (72 kDa) originate from a common precursor protein; they are secreted as a high density lipophorin (HDLp). ApoLp-III is synthesized and secreted as a distinct single protein with a molecular mass of 18 kDa. During development, apoLp-I and -II are present (as HDLp) in high amounts in IV- and V-instar larvae as well as in adult locusts. ApoLp-III is present primarily in the adult stage.

INTRODUCTION

Lipid transport in insects is performed by lipoproteins, which carry lipids from the site of absorption to the fat body and peripheral tissues. In resting locusts the major lipoprotein, present in the hemolymph, is high density lipophorin (reviewed by Beenakkers *et al.*, 1985). This HDLp is composed of two non-exchangeable apoproteins (apoLp-I and -II), phospholipid and diacylglycerol (DAG). Recently, association of a third small apoprotein (apoLp-III) with HDLp was reported (Surholt *et al.*, 1992). During insect flight, HDLp is loaded with large amounts of DAG at the fat body and simultaneously association of 14 molecules of apoLp-III occurs, resulting in the

formation of a low density lipophorin (LDLp) (reviewed by Van der Horst, 1990). After DAG hydrolysis at the flight muscles, LDLp dissociates into HDLp and apoLp-III in the hemolymph, which can be re-used for DAG loading and LDLp formation (shuttle function of HDLp). The synthesis of the protein parts of this lipid supply system (apoLp-I, -II and -III) by the fat body during locust development is described in the present study.

MATERIAL and METHODS

Preparation of hemolymph samples. Samples of hemolymph were taken from 3 female locusts (*Locusta migratoria*) of the same age group by a puncture in the ventral membrane between head and thorax using a Hamilton microsyringe, pooled and immediately diluted 1:1 (v/v) with insect saline (130 mM NaCl, 5 mM KCl, 1.9 mM NaH_2PO_4, 1.7 mM K_2HPO_4, pH 7.5) containing 10 mM EDTA to prevent blood clotting. Subsequently, saline containing 5 mM EDTA was added to a final hemolymph dilution of 1:14.

Electrophoresis. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Van der Horst *et al.* (1987). Samples of 10 µl diluted hemolymph were used. Protein bands were stained with Amido Black 10 B.

In vitro fat body incubations. Fat body tissue of 8-day old locusts was incubated *in vitro* in 250 µl Grace insect medium without leucine, supplemented with 10 µCi ^{3}H-leucine for 3 hr at 32 °C (Weers *et al.*, 1993).

Enzyme-linked immunosorbent assay (ELISA). Quantification of HDLp was carried out by the ELISA method as described (Weers *et al.*, 1992). ApoLp-III quantification by the ELISA method was carried out essentially as described for HDLp, except that monoclonal antibody 1F3 and rabbit polyclonal anti-apoLp-III serum were used. The production and the characteristics of the monoclonal antibodies have been described (Schulz *et al.*, 1987).

Immunoprecipitation was carried out essentially as described (Weers *et al.*, 1993), using monoclonal antibodies specific for apoLp-I, -II or -III (Schulz *et al.*, 1987).

RESULTS

Synthesis of apoLp-I, -II and -III by the fat body

When fat bodies were incubated *in vitro* in the presence of ^{3}H-labeled leucin, radiolabel was incorporated in newly synthesized proteins. Medium of such *in vitro* incubations was subjected to immunoprecipitation, using monoclonal antibodies specific for apoLp-I, -II or -III, and analyzed by SDS-PAGE and fluorography. Fig. 1 shows that all three apolipophorins were recovered as radiolabeled proteins, implying biosynthesis of these proteins. The immunoprecipitates using monoclonal antibodies 2E3 or 1E11 contained both apoLp-I and -II. However, when the 1F3 monoclonal antibody was used, only apoLp-III was recovered. This is in agreement with previous results demonstrating that apoLp-I and -II are synthesized and released as a lipoprotein particle (Weers *et al.*, 1992). The absence of apoLp-I and -II from 1F3 immunoprecipitates indicates that newly synthesized HDLp does not contain tightly associated apoLp-III.

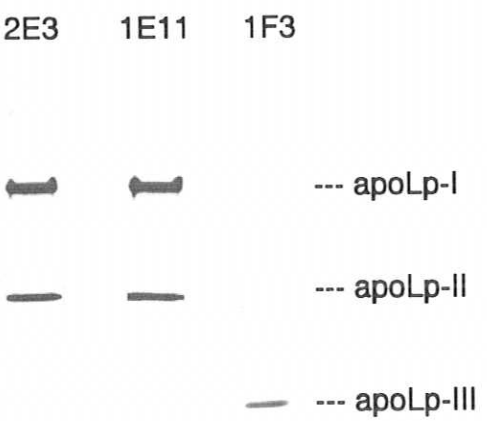

Fig. 1. Fluorogram of immunoprecipitates separated by 5-15% gradient SDS-PAGE. Media from fat body incubations were subjected to immunoprecipitation using the following monoclonal antibodies: 2E3 (anti-apoLp-I), 1E11 (anti-apoLp-II) and 1F3 (anti-apoLp-III).

Changes in the hemolymph pattern of apoLp-I, -II and -III during locust development.

When hemolymph samples, obtained from IV- and V-instar larvae and adult locusts, were subjected to SDS-PAGE and the gels were stained for protein, several changes in the hemolymph protein-pattern are observed. As shown in Fig. 2 apoLp-I is present in all three stages. ApoLp-II is present in the 75 kDa protein group and can not be discriminated from the other proteins in this group. However, since HDLp contains one single molecule of both apoLp-I and apoLp-II, changes in the apoLp-I pattern will correspond with those for apoLp-II. ApoLp-III (18 kDa) appeared to be present in larvae and young adults in very low amounts but an impressive increase of apoLp-III occurs approximately 5 days after adult ecdysis. Fig. 2. also shows the subunits of vitellogenin, most clearly in 11-days old adults.

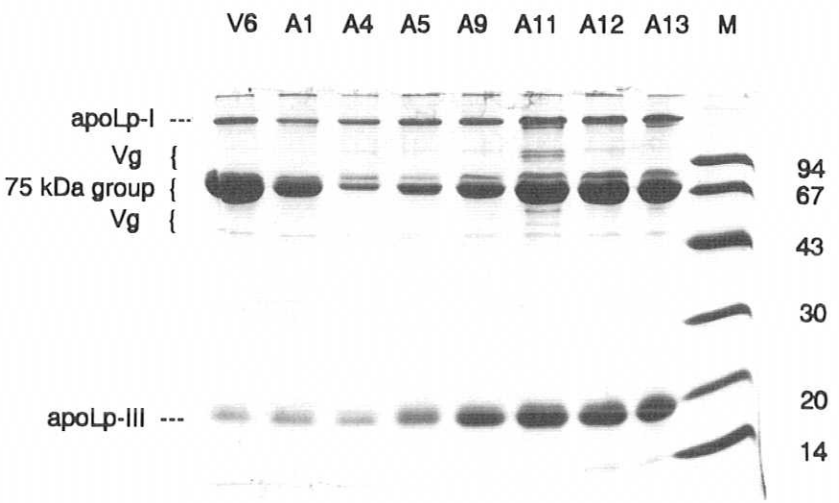

Fig. 2. SDS-PAGE of hemolymph proteins during female locust development. The numbers at the top represent the day after the last larval ecdysis (V) or imaginal ecdysis (A). The molecular masses (in kDa) of the marker proteins (M) are shown on the right. The 75 kDa group includes apoLp-II, cyanoprotein, larval storage protein and persistent storage protein (Wyatt et al., 1992).

The amounts of HDLp and apoLp-III were quantified by ELISA. From Table 1 it can be seen that HDLp is present in larvae as well as in adults. The concentration of HDLp increased approximately two-fold during the adult stage. A different pattern can be seen for apoLp-III: in larvae, only very low levels were obtained, whereas in the adult stage the concentration of apoLp-III reached values up to 17 mg/ml. It is clear that, in contrast to HDLp, the occurence of apoLp-III depends on the developmental stage.

Table 1. HDLp and apoLp-III concentrations in the hemolymph of locusts during development (n=5, concentrations in mg/ml hemolymph).

Stage	HDLp	apoLp-III
IV-instar larvae	7.2 ± 2.3	0.5 ± 0.2
V-instar larvae	8.4 ± 2.1	1.5 ± 0.7
3-days old adults	8.5 ± 2.4	2.0 ± 0.4
16-days old adults	17.7 ± 3.1	17.5 ± 3.7

DISCUSION

ApoLp-I, -II and -III are synthesized by the fat body as was clearly shown by immunoprecipitations of *in vitro* fat body incubations. ApoLp-I (220 kDa) and apoLp-II (72 kDa), which comprise the protein part of HDLp, are synthesized as a common precursor (Weers *et al.*, 1993). This high molecular weight precursor is cleaved into apoLp-I and -II, which are subsequently released as HDLp. An analogous synthesis mechanism has been proposed for vitellogenin of the mosquito *Aedes aegypti* (Bose and Raikhel, 1988). This female specific yolk protein is synthesized as a high molecular weight protein which is cleaved into subunits of 65 and 200 kDa (Dhadialla and Raikhel, 1990).

During locust development, HDLp is present in IV-, and V-instar larvae, and in adults. The concentration of HDLp increased from 8 mg/ml in larvae to 17 mg/ml in older adults. ApoLp-III was almost absent in larvae, but increased tremendously during adult development. This reflects the role of apoLp-III during insect flight in

adult locusts: association with HDLp and DAG resulting in the formation of LDLp which is capable to transport high amounts of lipid to the energy demanding flight muscles (Van der Horst, 1990). The initiation of the apoLp-III synthesis during adult development will be a fascinating subject for further investigation.

REFERENCES

Beenakkers A.M.Th., D.J. Van der Horst & W.J.A. Van Marrewijk, 1985 - Insect lipids and lipoproteins and their role in physiological processes. *Prog. Lipid Res.* **24,** 19-67.

Bose S.G. & A.S. Raikhel, 1988 - Mosquito vitellogenin subunits originate from a common precursor. *Biochem. Biophys. Res. Commun.* **155,** 436-442.

Dhadialla T.S. & A.S. Raikhel, 1990 - Biosynthesis of mosquito vitellogenin. *J. Biol. Chem.* **265,** 9924-9933.

Schulz T.K.F., D.J. Van der Horst, H. Amesz, H.O. Voorma & A.M.Th. Beenakkers, 1987 - Monoclonal antibodies specific for apoproteins of lipophorins from the migratory locust. *Arch. Insect Biochem. Physiol.* **6,** 97-107.

Surholt B., J.M. Van Doorn, J. Goldberg & D.J. Van der Horst, 1992 - Compositional analysis of high- and low-density lipophorin of *Acherontia atropos* and *Locusta migratoria*. *Biol. Chem. Hoppe-Seyler* **373,** 13-20.

Van der Horst D.J. (1990) Lipid transport function of lipoproteins in flying insects. *Biochim. Biophys. Acta.* **1047,** 195-211.

Van der Horst D.J., A.M.Th. Beenakkers, J.M. Van Doorn, K. Gerritse & T.K.F. Schulz (1987) Adipokinetic hormone-induced lipid mobilization and lipophorin interconversions in fifth larval instar locusts. *Insect Biochem.* **17,** 799-808.

Weers P.M.M., D.J. Van der Horst, W.J.A. Van Marrewijk, M. Van den Eijnden, J.M. Van Doorn & A.M.Th. Beenakkers, 1992 - Biosynthesis and secretion of insect lipoprotein. *J. Lipid Res.* **33,** 485-491.

Weers P.M.M., W.J.A. Van Marrewijk, A.M.Th. Beenakkers & D.J. Van der Horst, 1993 - Biosynthesis of locust lipophorin: apolipophorin I and II originate from a common precursor. *J. Biol. Chem.* In press.

Wyatt G.R., M.R. Kanost, B.C. Chin, K.E. Cook, B.M. Kawasoe & J. Zhang, 1992 - Juvenile hormone analog and injection effects on locust hemolymph protein synthesis. *Arch. Insect Biochem. Physiol.* **20,** 167-180.

PROCTOLIN-MEDIATED CONTROL OF HINDGUT CONTRACTIONS IN THE COLORADO POTATO BEETLE: IMMUNOHISTOCHEMISTRY AND NOVEL BIOASSAY STUDIES

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Key words: *Leptinotarsa decemlineata*, Colorado potato beetle, proctolin, impedance converter, hindgut

Summary.

Immunohistochemical studies with an anti-proctolin antibody revealed the presence of proctolinergic neurons in the ventral nerve cord, innervating the hindgut musculature either directly or indirectly. The stimulatory action of proctolin on contraction rhythm was measured electronically with an impedance converter.

INTRODUCTION

The insect hindgut has been the subject of many studies on the complex action and interaction of neuroactive substances on gut contraction. Several peptides with myotropic activity have been identified that affect hindgut contractions in one way or another. Proctolin, originally identified by Starratt & Brown (1975), is among the peptides which give a stimulation of neurally induced hindgut contractions. It is considered to act as a neuromodulator (Cook & Holman, 1979). Many other myotropic substances were discovered by testing hormone samples on cockroach guts. In such assays, isolated hindguts under saline are attached vertically to a mechanical transducer and physical contractions are displayed on a chart recorder (Holman et al., 1991).

The gut of the Colorado potato beetle (*Leptinotarsa decemlineata*) is of interest to us for analyzing peptidergic and aminergic control mechanisms. It appears to be under a dual control, i.e. by the central as well as the stomatogastric nervous system, as followed from immunohistochemical studies. Serotonin (van haeften and Schooneveld, 1992; van Haeften et al., 1993) and an FMRFamide-related peptide are among the neurochemicals that are closely associated with the gut musculature (Schooneveld et al., 1992). A proctolin-immunoreactive substance has been observed in the central and peripheral nervous system, and in the nerves running to the alimentary tract (Veenstra et al., 1985).

We studied the proctolinergic system innervating the hindgut by means of immunocytochemistry and developed a novel bioassay to determine the role of synthetic proctolin on contractions of the fragile hindgut.

MATERIALS AND METHODS

Animals. We used long-day beetles of 1 week old, bred in the laboratory. Guts were dissected under saline and care was taken not to apply tension to guts to be used for the bioassay.

Immunohistochemistry. Guts used for wholemount immunohistochemistry were fixed in 4% formaldehyde solution in a phosphate buffer pH 7.3 for 6 hrs, rinsed, and incubated with rabbit anti-proctolin serum (generously supplied by Professor M. O'Shea), diluted 1:500 in PBS with Triton X-100 (0.25%) and NaN_3 (0.1%) for 48 hrs 4°C. Ventral nerve cords were fixed for 6 hrs in GPA, embedded in paraplast, and sectioned at 6 m. Sections were immunostained overnight in the same antibody, at 4°C. We used the indirect enzymatic peroxidase method to detect the immunolabel, with diaminobenzidine as the chromogen.

Bioassay procedure. The carefully dissected guts were placed in saline (Khan et al., 1982) in series of six. About half of the preparations spontaneously started coiling and contracting and continued showing this motility for several hours, while the others soon became immobile. The latter guts were taken for next tests in the bioassay instrument (Fig. 3) and were reduced in size to study the motility of the rectum in the recording chamber. Insect pins were placed through the remaining part of the ileum and through the anal muscles, such that the rectum was kept free from the bottom of the chamber.

The recording chamber was a shallow depression in a Sylgard block, which was filled with 1 ml of saline. To avoid the possibility that proctolin in the low

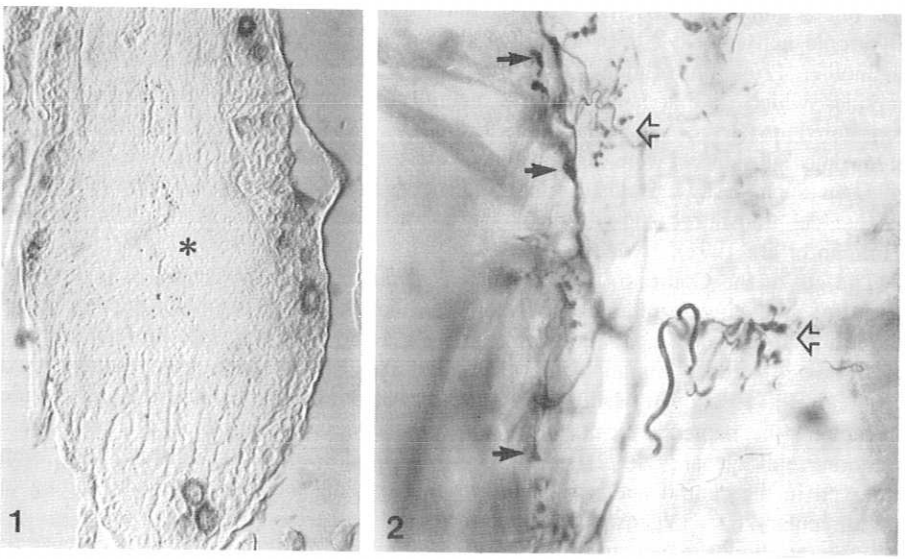

Fig. 1. Paraffin section of last abdominal ganglion showing proctolin-containing neurons at theperiphery and axon swellings in neuropil (asterisks). x200.
Fig. 2. Proctolinergic axons running over longitudinal muscles on rectum, showing bead-like swellings indicative of neurohemal sites (arrows); branches over circular muscles show arborizations ending in clusters of synaptic boutons (thick arrows). x333.

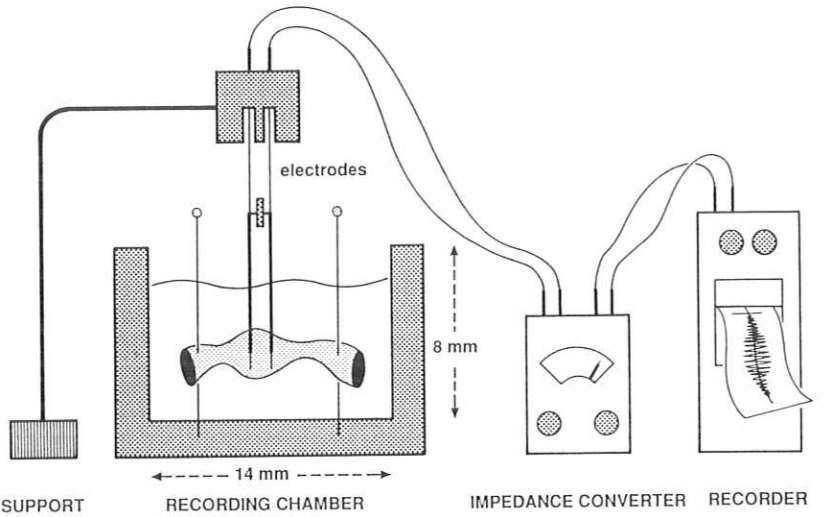

Fig. 3. Experimental set-up for monitoring hindgut contractions. An adjustable set of electrodes that are positioned close to the most contractile part of the rectum. The hindgut is mounted in a bath filled with saline and can move freely between the pins that holds it free from the bottom. Changes in impedance near the contractile tissue are monitored by an impedance converter and the outgoing DC signals fed into a Brush chart recorder. Instruments not drawn to scale.

concentrations would stick to the surfaces of apparatus and tissues, 0.1% BSA was added. All test dilutions were made in PBS. Test solutions were administered directly to the bath by pipette in a 1 ml volume, after first removing the same volume from the bath. The assay was terminated by several rinses of PBS.

Impedance recording. The contracting gut causes a change in impedance of the field between electrodes that were placed close to the gut in a strategic position: where displacements of tissue during contractions are maximal. The size of the field is determined by the distance of the electrodes (typically 1 mm) and the length of the conducting electrode surface. The electrode is a stainless steel wire of 2.7 cm long, insulated with nail polish over its entire surface except for the extreme tip. The change in impedance (a mix of changes in resistance, capacitance, and inductance) is monitored through a 50 kHz alternating voltage, generated by an oscillator of an impedance converter (Biocom Inc., model 2991, Culver City, CA) which modulates the current changes into a shift in the outgoing DC signal. The DC signal is fed into a chart recorder (Gould Inc. model Brush 220, Cleveland Ohio). Gut contractions are thus visualized as graphs with peak intervals that correspond to contraction frequency. The height of the peaks is grossly, not linearly, related to the force of contraction. The relative heights are related to relative contraction forces of a given gut preparation. Visible gut movements always caused a peak in the recording, and due to the sensitivity of the instrument, contractions could thus be recorded electronically that were too small to be seen.

RESULTS

Immunohistochemistry

The proctolinergic system in the ventral nerve cord consists of a number of bilaterally arranged neurons (Fig. 1) which appear to have more than one axon branch: one running into the neuropil, the other entering one of the abdominal nerves. Two of the abdominal nerves innervate the hindgut and divide several times: (1) nerves running along longitudinally arranged muscles develop swellings which have the appearance of neurohaemal thickenings; (2) nerves addressing the transverse circular muscles develop fine arborizations with tiny terminal swellings which have the appearance of presynaptic structures (Fig. 2).

Nature of gut contractions

Hindguts mounted in our bioassay chamber may make both peristaltic and coiling movements, as a result of the non- synchronized contraction of the longitudinal and circular muscles. These movements are reflected in the complex patterns in DC voltage output recorded. Unstimulated assay guts usually relaxed after a while, and when proctolin was added a rather simple spike pattern occurred as a result of contractions of especially the longitudinal muscles. Rinsing the preparation several times with fresh saline reinstalled a relaxation.

Action of proctolin

The addition of proctolin stimulates both contraction frequency and amplitude and the reactions are clearly dose-dependent (Fig. 4). Individual guts differ in sensitivity, the lowest threshold concentration recorded was 10^{-10}M, but the threshold most often determined was 10^{-8}M. Responses were scored positive only in as far as a reaction in either frequency or amplitude was seen within a minute after the application of proctolin and that lasted for

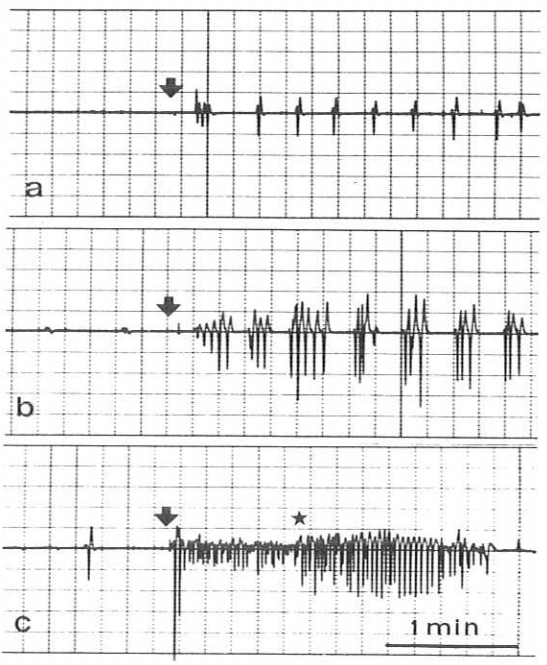

Fig. 4. Series of recordings of dose-dependent reactions of a hindgut typically stimulated by exposure to synthetic proctolin (strips a-c); A dose of 10^{-8}M induces a regular contraction pattern within seconds (a). *Both contraction frequency and amplitude increase with a dose of 10^{-7}M* (b), *whereas 10^{-6}M causes a tetanus contraction with a total standstill after 2.5 min* (c; *at asterisk, recorder sensitivity was changed to reveal shape of spikes).*

at least one minute. Dosages up to a factor 100 above threshold value cause a state of contraction without full relaxation (Fig. 4).

DISCUSSION

The hindgut is a complex organ; it is responsible for the caudal movement of the gut contents, for water withdrawal from the fecal pellet, and for defecation. It is conceivable that different physiological aspects are controlled in different ways and with the cooperation of different sets of muscles. Proctolin is only one of the neurochemical mediators involved. Axons containing FMRFamide-like substances were observed by Schooneveld et al. (1992) and also serotonin was seen to innervate the hindgut muscles (van Haeften et al. 1993). We speculate that an extended immunohistochemical screening with antibodies against other regulatory neurochemicals may well reveal additional innervation pathways. Cantera and Nassel (1991) revealed some of such complexity in the blowfly.

This study shows that proctolin has a stimulatory action on hindgut contraction when used in concentrations (10^{-8}M and lower) that can be considered physiological. Still, proctolin is not utilized in a simple way. We showed that the longitudinal muscles carry axon thickenings resembling release sites for proctolin, whereas the circular muscles branch heavily and seem to represent a diffuse web of presynaptic swellings. We may, therefore, infer that the longitudinal muscles are addressed by proctolin, released locally as a neurohormone, whereas the circular muscles seem to be addressed synaptically. The physiological relevance of this setup remains te be determined. With regard to this organ system, proctolin may thus act both as a neurohormone and neurotransmitter or modulator.

The measurement of contraction activity was facilitated considerably by the introduction of our impedance converter-based instrumentation. It allowed the transformation of even minute movements of tissue into recorder tracings. Although the method of quantification is not strictly accurate, it can be utilized with great advantage over the traditional methods for recording contraction performance: (1) the organs are left unrestrained, no tension is applied and sensitivity does not change over a period of hours; (2) activation of different sets of muscles can be recorded: the tracings of peristaltic contractions differ from the coiling movements, which helps interpreting the effects; (3) there is no lower limit as to the size of the contractile organ to be investigatred. Moreover, several assays can be done with the same preparation and within a matter of minutes, depending on the assay conditions.

We anticipate a more extensive series of experiments to evaluate the effects of other candidate-modulatory compounds. We also plan to perform similar contraction studies with organs that are substantially smaller and more fragile than beetle hindgut.

REFERENCES

CANTERA, R. & NASSEL, D.L. (1991) Dual peptidergic innervation of the blowfly hindgut: A light- and electron microscopic study of FMRFamide and proctolin immunoreactive fibers. *Comp. Biochem. Physiol.* **99C,** 517-525.

COOK, B.J. & HOLMAN, G.M. (1979) The pharmacology of insect visceral muscle. *Comp. Biochem. Physiol.* **64C**, 183-190.

HOLMAN, G.M., NACHMAN, R.J., SCHOOFS, L., HAYES, T.K., WRIGHT, M.S. & DE LOOF, A. (1991) The *Leucophaea maderae* hindgut preparation: a rapid and sensitive bioassay tool for the insect myotropins of other insect species. *Insect Biochem.* **21**, 107-112.

KHAN, M.A., DODERER, A., KOOPMANSCHAP, A.B., & DE KORT, C.A.D. (1982). Improved assay conditions for measurement of corpus allatum activity in vitro in the Colorado potato beetle, *Leptinotarsa decemlineata. J. Insect Physiol.* **28,** 279-284.

SCHOONEVELD, H., SMID, H.M., AMMERLAAN, W., & VAN HELDEN, J.M. (1992) Colocalized FMRFamide-related neuropeptides in nervous system of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera, Chrysomelidae) demonstrated immunohistochemically with mono- and polyclonal antibodies. **Int. J.** *Insect Morphol. Embryol.* **21,** 285-298.

STARRETT, A.N. & BROWN, B.E. (1975) Structure of the pentapeptide proctolin, a proposed neurotransmitter in insects. *Life Sci.***17**, 1253- 1256.

VAN HAEFTEN, T. & SCHOONEVELD, H. (1992) Serotonin-like immunoreactivity in the ventral nerve cord of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say): Identification of five different neuron classes. *Cell Tissue Res.* **270**, 405-413.

VAN HAEFTEN, T., SMID, H.M. and SCHOONEVELD, H. (1993). Serotoninergic innervation of the alimentary tract of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say): Structural and functional aspects. Cell Tisue Res. (in press).

VEENSTRA, J., ROMBERG-PRIVEE, H.M., and SCHOONEVELD, H. (1985). A proctolin-like peptide and its immunocytochemical localization in the Colorado potato beetle, *Leptinotarsa decemlineata. Cell Tissue Res.* **240**, 535-540.

FIELD AND LABORATORY SINGLE CELL RECORDINGS OF PHEROMONE RECEPTORS OF *ADOXOPHYES ORANA* AND *PANDEMIS HEPARANA*

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Key words: *Adoxophyes orana*, *Pandemis heparana*, single cell recording, pheromone plume, pheromone dispersion, gas chromatography

Summary

Recordings were made from individual pheromone receptor cells on the antennae of males of two tortricid moth species in an apple orchard treated with commercial pheromone dispensers for mating disruption and in an open terrain, in which artificial pheromone sources were placed. The results demonstrate the ability of the moths to discriminate discrete pheromone plumes from an homogeneous background. Comparison between the response intensities recorded in the field and those evoked by samples from a gas chromatograph reveals that the responses recorded in the field are evoked by concentrations in the order of 100 - 200 pg/l of Z11-14:Ac.

INTRODUCTION

Recent research on pheromone signal transmission in moths and its behavioural effect has revealed that, in addition to the chemical composition of the pheromone blend, the distribution of the pheromone molecules in the plume is of crucial importance.

Proper orientation towards a pheromone source is obviously mediated by a characteristic pattern of the pheromone plume in space and time. Direct observations of orientation in a pheromone plume have mainly been carried out in wind tunnels. However, due to the rather artificial conditions maintained in wind tunnels, the results cannot simply be extrapolated to the situation in the field. Field measurements of pheromone plume structure can only be accomplished by using the insect's pheromone receptors as a detector. No other detectors are available combining the required high levels of and sensitivity and selectivity. Two standard electrophysiological methods for recording responses of antennal pheromone receptor cells under laboratory conditions are available: electroantannography (EAG) and single sensillum recording (SSR). The EAG technique has been applied in the field by Baker & Haynes (1989), and a portable EAG recording device was constructed by Koch (1990) and used for pheromone concentration measurements in the field (Sauer et al, 1992). Although the EAG technique might be well suited for concentration measuring, it lacks a sufficiently high temporal resolution, which is required for the study of fast concentration fluctuations such as those occurring in pheromone plumes.

Therefore, we accommodated the SSR technique to operation in the field by using a specially constructed portable recording device (Van Der Pers & Minks, 1993). The device enables recording of both activity from individual pheromone receptor cells and the air

velocity in close proximity of the recording site; the latter by means of a fast responding thermistor air flow sensor. Only the combined response of the receptor cell and the air flow sensor allows a proper analysis of pheromone plume structure and pheromone perception by moths in the field. The field recordings are compared to SSR obtained by stimulating the pheromone receptors with precisely defined pheromone-air mixtures from the effluent of a gas chromatograph (GC) by means of coupled GC-SSR.

The aim of these studies is to gain fundamental understanding of the factors affecting pheromone communication under natural conditions. Although the practical execution of SSR in the field is seriously hindered by the unpredictable and uncontrollable outdour conditions, just these factors need further examination.

MATERIALS AND METHODS

The recordings were made from antennal sensilla of males of *Adoxophyes orana* (F.v.R.) and *Pandemis heparana* (Den. & Schiffm.)(Lepidoptera; Tortricidae), obtained from a rearing stock. The portable recording module (constructed by SYNTECH, Hilversum, The Netherlands) is a compact combination of the essential elements necessary for SSR and simultaneous air velocity measurement. Constructional details are given in Van Der Pers & Minks (1993). The SSR recording technique is similar to the tip recording method described by Van Der Pers & Den Otter (1978), but with tungsten instead of glass micro-knives. The signals were stored on a cassette tape recorder.

Disposable syringes containing a filter paper loaded with 10μg of (Z)-11-tetradecen-1-ol acetate (Z11-14:Ac) were used for testing the responsiveness of the preparations. Z11-14:Ac is a major sex pheromone component in both *A. orana* and *P. heparana*. Syringes loaded with 5000 μg Z11-14:Ac were used in artificial pheromone sources to be set out in the field. Each of these sources consisted of a small electric membrane air pump (delivery 10 ml/s), the outlet of which was connected to the syringe containing Z11-14:Ac through an activated charcoal filter. The whole apparatus was attached to a post, thus enabling the device to be placed anywhere in the ground. The outlet of the syringe was about 1 meter above ground level. Pheromone delivery from these sources was remotely controlled by switching the air pumps on or off. The recording module was positioned under a stereo dissecting microscope placed on a camping table.

Recordings were made under two different conditions. A first set of recordings was made in an apple orchard where a grid of pheromone dispensers had been installed. A second series of recordings was made in an open field with 5 pheromone sources placed at a distance of 7 meters upwind of the preparation and 2 meters apart.

Laboratory recordings were made with the same recording module as was used in the field and by means of the same technique. During the recordings the antennal preparation was positioned at a distance of 5 mm from the outlet of a PTFE tube. A continuous flow of charcoal filtered and humidified air was applied through the tube at a velocity of 1 m/s over the preparation. The effluent from a micro needle-valve split inside the gas chromatograph (Chrompack CP9000) was led through a heated (200^{0}C) transfer line and mixed into the continuous air flow. The split valve was inserted between the column (Chrompack WCOT fused silica 10m x 0.25 mm coated with CP-Sil 5CB)and the flame ionization detector (FID) to obtain an additional outlet for the SSR module. The split ratio between the FID and the SSR outlet was 2:1. Samples (1 μl) of 0.05 - 10 ng Z11-14:Ac dissolved in hexane were injected splitless. Helium was used as carrier gas. A temperature rise (100 - 150 ^{0}C) was programmed to obtain a relative short retention time (4.8 minutes) of Z11-14:Ac . The FID

signal was recorded simultaneously with the action potentials from the SSR module on an instrumentation tape recorder. The latter signal was subsequently converted to a voltage proportional to the number of actionpotentials per second by a level discriminator followed by frequency to voltage converter.

RESULTS

The recordings from pheromone receptors of male *A. orana* made in the apple orchard all showed a relative high activity during exposure to abient air (Fig.1). This activity was decreased during periods in which the recording module was shielded from ambient air currents by means of a plexiglass hood (Van Der Pers & Minks, 1993). During exposure to ambient air, the activity of the receptors was partly related to the momentary air velocity, as can be seen in Fig.1. An increase in air velocity was often followed by an increase in the response intensity of the receptor cell.

Recordings in an open field, where the preparation was exposed to a row of 5 pheromone sources placed at 7 meters upwind, were obtained from antennal pheromone receptors of male *P. heparana*. Recording periods during which the pheromone sources were active were alternated with periods in which the pheromone sources were switched off. During periods of inactive pheromone sources, the receptors showed a moderate level of spontaneous activity. Shortly after activation of the pheromone sources the receptors produced bursts of increased firing frequency (Fig.3).

Fig.3 also shows that there was little or no relation to the air velocity. The cell's firing rate during activity bursts was about 10 spikes/s. The firing pattern of shorter and longer activity bursts during exposure to the plumes emitted by the pheromone sources was characteristic for all the recordings made under these conditions.

A typical example of a recording from a pheromone receptor of a male *A.orana* in response to a sample of Z11-14:Ac eluting from the gas chromatograph is presented in Fig.2. The slight delay in response was caused by the difference in flow rate after the split valve. The elution peak width of the samples was consistently 4 to 4.5 s, independent of the amount injected. The elution profile of the Z11-14:Ac sample and the intensity profile of the responding receptor cell were almost identical. In all recordings, the total response of the receptor was proportional to the amount of Z11-14:Ac eluting, ranging from a few spikes in response to 50pg, 10 spikes to 100 - 150 pg to about 350 evoked by 3ng. Similar results were obtained from recordings of *P. heparana* receptors.

DISCUSSION

Knowledge about the concentration and the dispersion of pheromones introduced in the field for mating disruption is crucial for an appropriate evaluation of the effectiveness of this method. Although an average pheromone concentration could be calculated on the basis of emission rates of the pheromone dispensers and their density, these calculations are inevitably not more that a crude estimate due to the highly fluctuating atmospheric conditions in the field. Sauer et al (1992) measured EAG responses from antennae of male *Lobesia botrana* in vineyards treated with pheromone for mating disruption and in areas free of artificial pheromone sources. The EAG responses showed a drastic decline in pheromone concentration at increasing distances from the edge of the treated vineyard. However, the concentrations are expressed in relative units rather than in absolute values.

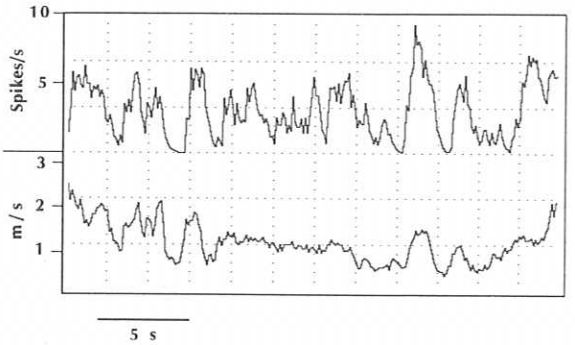

Fig.1 Recording of pheromone receptor cell activity of male A.orana (upper trace) (derived from the instantaneous firing rate by frequency to voltage conversion) and air velocity (lower trace) in an apple orchard supplied with dispensers for mating disruption.

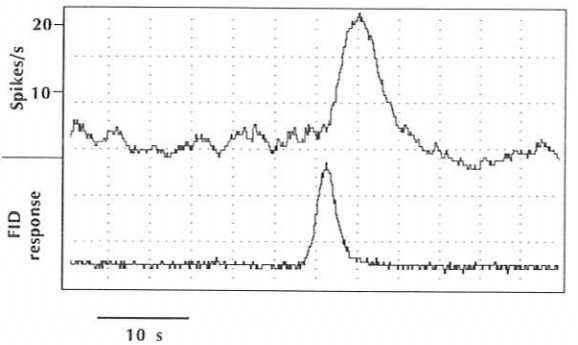

Fig.2 Recording of pheromone receptor cell activity of male A.orana (upper trace) (derived from the instantaneous firing rate by frequency to voltage conversion) and FID response (lower trace) during elution of 250 pg Z11-14:Ac.

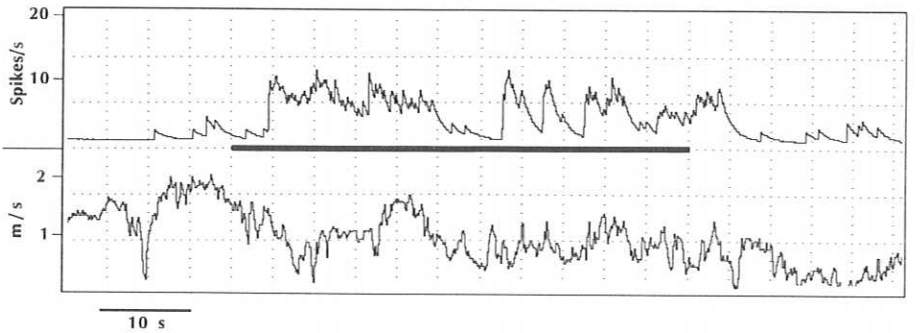

Fig.3 Recording of pheromone receptor cell activity of male P.heparana (upper trace) (derived from the instantaneous firing rate by frequency to voltage conversion) and air velocity (lower trace) in an open terrain at a distance of 7 meters downwind from artificial sources loaded with Z11-14:Ac.
Black horizontal bar indicates period during which artificial pheromone sources were in operation.

Our GC-SSR experiments with *A. orana* and *P. heparana* indicated that an amount of 50 pg of Z11-14:Ac is just enough to elicit a few action potentials. The sample is applied to the antenna in a constant air flow during an elution period of 4.5 s. The flux (defined by Kaissling, 1990, as the product of concentration and air velocity) over the antenna begins at zero, reaches a maximum after 2.2 s and returns to zero after 4.5 s. Given a flow rate of 1 m/s through a tube of 7 mm inner diameter, a sample of 50 pg corresponds to an average concentration of 144 pg/l during the stimulation period of 4.5 seconds. The activity recorded during measurements in a pheromone-treated orchard is in the range of 0 - 9 spikes/s (Fig.1). Comparing this value to the GC-SSR data reveals that in the field situation the sensillum is stimulated with average concentrations in the same order of 144 pg/l. In both the GC-SSR experiments and the field recordings the time course of the responses reflects the increase and decrease in the flux over the antenna (Fig.1 and 2). In the GC-SSR measurements, in which the air velocity over the antenna is fixed at 1m/s, this fluctuation is due to the profile of the elution peak, whereas in the pheromone treated orchard, in which the distribution of pheromone is rather homogeneous, this is caused by fluctuations in ambient air velocity (Fig.1 lower trace).

Preliminary experiments aimed at recording activity from a distant artificial pheromone source taught that the likelihood of the plume to strike the antennal preparation was very small, a difficulty also encountered by Baker & Haynes (1989) during their field EAG experiments. For this reason we used 5 artificial odour sources at a distance of 7 meters from the preparation and 2 meters apart, in order to increase the probability of the plume hitting the antenna.

The recordings of *P.heparana* pheromone receptors to stimulation with artificial pheromone sources in an open terrain show bursts of receptor activity during operation of the sources. The activity during the bursts is in the range of 5 - 10 spikes/s, indicating that the amounts of pheromone during the bursts are in the range of 50 - 150 pg. In this situation the variations were not correlated with air velocity fluctuations. Consequently, the responses reflect differences in pheromone flux due to differences in concentration inside and outside the pheromone plume.

The antenna of *A. orana* and *P. heparana* are provided with receptors sensitive to air speed fluctuations. The combined information of these receptors and the responses from the pheromone receptors enables the insects to differentiate between ambient air containing a homogeneous pheromone distribution and the irregular distribution in a plume. This ability is clearly illustrated by behavioural experiments. Kennedy (1982) demonstrated that male *A.orana* moths do not show searching behaviour upon stimulation with a uniform pheromone cloud. Baker et al (1985) reported that male oriental fruit moths, *Grapholita molesta*, do not fly upwind in a uniform cloud of pheromone, but readily do so in a pulsed cloud or in a plume. Upwind flight is also initiated if a plume is superimposed on a continuous background (Baker et al, 1985). In experiments with the cabbage looper moth, *Trichoplusia ni*, Liu & Haynes (1992) demonstrate that males of this species are able to detect a pheromone plume in a background noise containing a uniform distribution of an inhibitor for the pheromone of this species.

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REFERENCES

BAKER, T.C. & K.F. HAYNES, 1989 - Field and laboratory electroantennographic measurements of pheromone plume structure correlated with oriental fruit moth behaviour. *Physiological Entomology*, **14**, 1-12.

KAISSLING, K.-E., 1990 - Sensory basis of pheromone-mediated orientation in moths. *Verh. Dtsch. Zool. Ges.* **83**, 109-131.

KENNEDY, J.S., 1982 - Mechanism of moth sex attraction: a modified view based on wind-tunnel experiments with flying male *Adoxophyes*. *Coll. INRA* **7**, 189-192.

KOCH, U.T., 1990 - A portable EAG system for measurement of pheromones in the field. In: *Abstracts 200th ACS National Meeting*, Washington, D.C. August 26-31, 1990; AGFD 31.

LIU, Y-B. & K.F. HAYNES, 1992 - Filamentous nature of pheromone plumes protects integrity of signal from background chemical noise in cabbage looper moth, *Trichoplusia ni*. *J. Chem. Ecol.* **18**, 299-307.

SAUER, A.E., G. KARG, U.T. KOCH & J.J. DE KRAMER, 1992 - A portable EAG system for the measurement of pheromone concentrations in the field. *Chemical Senses*. **17,** 543-553.

VAN DER PERS, J.N.C. & A.K. MINKS, 1993 - Pheromone monitoring in the field using single sensillum recording. *Entom. exp. appl.* (in press)

VAN DER PERS, J.N.C. & C.J. DEN OTTER, 1978 - Single cell responses from olfactory receptors of small ermine moths to sex-attractants. *J. Insect Physiol.* **24**, 337-343.

EFFECT OF PHOTOPERIOD ON DIAPAUSE DURATION IN THE TWO-SPOTTED SPIDER MITE (*Tetranychus urticae* Koch)

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Key words:
diapause, *Tetranychus urticae*, quantitative photoperiod perception, photoperiodism

Summary

In two different laboratory experiments the effect of photoperiod on diapause duration in the spider mite *Tetranychus urticae* was examined. Both experiments revealed no effect on mean duration of diapause. However, in the second experiment a clear influence on the amount of within-treatment variation was found: when mites were exposed to photoperiods with dark phases close to the so-called critical nightlength, diapause completion was more synchronized.

INTRODUCTION

Once insects and mites have entered diapause they cannot leave this state instantly in reaction to more favourable conditions. First a dynamic physiological process called 'diapause development' has to be completed. The rate of diapause development often appears to be under photoperiodic control (Tauber & Tauber, 1976; Tauber *et al.*, 1986; Danks, 1987). Although the exact physiological mechanism of diapause induction and development is still far from understood, general consensus exists that one important component should be a photoperiodic clock (Saunders, 1981). Such a clock measures the duration of light- and/or dark-phases of photoperiodic cycles.

To date, it is unknown how exactly photoperiodic time measurement is performed, but basically there are two possibilities: qualitatively or quantitatively. In qualitative time measurement the clock simply assesses whether, for example, nights are longer or shorter than some critical nightlength (Lees, 1981; Pittendrigh, 1981; Vaz Nunes & Veerman, 1982; Lewis & Saunders, 1987). By definition, the critical nightlength is the threshold nightlength at which 50% of individuals respond, either during induction or

termination of diapause (*sensu* Danks, 1987). In quantitative time measurement the clock assesses the absolute nightlength (Tauber & Tauber, 1973; Zaslavski & Fomenko, 1983; Zaslavski, 1988; Hardie, 1990; Spieth & Sauer, 1991). Here we report on part of a study that aims at determining whether photoperiodic time measurement is qualitative or quantitative in the two-spotted spider mite, *Tetranychus urticae* Koch.

One way of studying the photoperiodic clock is through exposure of groups of animals to different photoperiods, either during pre-diapause development or during diapause development, and assess diapause intensity. If time were measured quantitatively, it could be possible that different photoperiods at one side of the critical threshold result in different diapause intensity (*e.g.* expressed as mean diapause duration). If time were measured qualitatively, such a correlation between the absolute length of one phase of a photoperiod and diapause intensity would be much weaker or even absent.

The absolute length of a certain phase of a photoperiod can be important at different instances during the diapause process. Some studies report an effect on diapause duration of the nightlength of the diapause induction regime (Bell & Adkisson, 1964; Numata & Hidaka, 1983; Beck, 1989). Others report effects on diapause duration of the nightlength of the so-called 'termination regime', *i.e.* the regime at which diapause development takes place (Tauber & Tauber, 1973; Gomi & Takeda, 1992). The general conclusion of both groups of studies is that diapause duration may quantitatively depend on photoperiod.

In the temperate climate zones, *T. urticae* exhibits a facultative reproductive diapause which is induced by so-called long-night photoperiods (Veerman, 1985). Critical nightlength and diapause intensity depend on geographic origin (Vaz Nunes *et al.*, 1990; Koveos *et al.*, 1993). Diapause is only expressed in females; males do not survive the winter period. Characteristics of diapausing females are an orange-red colour and absence of reproduction (Veerman, 1985).

Here, the effects of photoperiod on diapause duration in *T. urticae* females were studied in two experiments:

1. Effect of photoperiod of induction regime

Groups of mites were exposed to different diapause inducing photoperiods. After induction of diapause they were transferred to the same termination regime.

2. Effect of photoperiod of termination regime

After induction of diapause at one regime, groups of mites were transferred to termination regimes with different long-night photoperiods.

In both experiments the responses of individual mites were studied (*cf.* Kroon & Veerman, 1991), and diapause duration was taken as a measure of diapause intensity.

MATERIALS AND METHODS

Mites

A dutch strain of *T. urticae* was reared in the laboratory on bean plants (*Phaseolus vulgaris* L.), ever since 1961. For this study an inbred line was obtained by sib-selection (20 generations), to keep variation in photoperiodic response as small as possible. Stock mites were kept at 22°C, under long-day photoperiods (LD17:7h). For the experiments female mites were allowed to lay eggs for 24 hours on detached bean leaves (25-30 mites/leaf), at 26°C and continuous illumination. After three days at this regime the eggs were ready to hatch. Leaves with eggs were then transferred to the diapause inducing regimes, at which development was completed. After diapause expression the females were stored at 4°C. The critical nightlength, at 19°C, of the strain used in this study is 10 hours (LD 14:10h; Veerman, 1977b).

In both experiments diapause was terminated under long-night photoperiods. In mites kept under long nights, diapause development proceeds slowly and eventually ends spontaneously (Veerman, 1977a; Hodek, 1983; Koveos *et al.*, 1993).

Experiment 1: Effect of photoperiod of induction regime on diapause duration

Diapause was induced at four regimes: LD12:12, 10:14, 8:16 or 6:18h, all at 19°C. Diapausing females were stored for three weeks at 4°C. Then they were put on bean leaf discs (192 females/treatment, 1 female/disc) and transferred to one termination regime (LD10:14h, 19°C).

Experiment 2: Effect of photoperiod of termination regime on diapause duration

Diapause was induced at LD10:14h, 19°C. After diapause expression the females were stored for three weeks at 4°C. Then they were transferred to one of four termination regimes: LD12:12, 10:14, 8:16 or 6:18h, all at 19°C (192 females/treatment, 1 female/ leaf disc).

In both experiments all females were observed thrice a week. Appearence of the first egg(s) was taken as the sign of diapause termination. When necessary deteriorated leaf discs were replaced by fresh ones.

RESULTS

1. Effect of photoperiod of induction regime on diapause duration

On average, diapause duration for all treatments was about 60 days, ranging from 59.9 to 61.8, for the induction regimes LD10:14 and LD12:12h respectively. Thus the different induction regimes did not result in significantly different intensity of diapause.

2. Effect of photoperiod of termination regime on diapause duration

Again, no significant differences in mean duration of diapause were found between the four treatments. On average, diapause duration was about 65 days, ranging from 62.2 to 66.4, for the termination regimes LD12:12 and LD10:14h respectively.

However, the amount of within-treatment variation appeared to be significantly different between treatments, and was correlated to the length of the night phase of the photoperiod. The longer the nigth phase of the termination regime, the higher the variance, ranging from 127 (LD12:12h) to 719 (LD6:18h).

In conclusion, photoperiods of the termination regime do not seem to affect mean diapause duration, but there does seem to be an effect on the amount of variation.

DISCUSSION

Koveos *et al.* (1993) found an effect of photoperiod of the regime at which diapause was terminated on diapause duration. In their experiments they exposed a number of geographic strains of *T. urticae* to two termination regimes, *viz.* LD17:7 and LD10:14h (19°C). The dark phases of the first regime were shorter than the critical nightlength, those of the second regime were longer. It was found for all strains that the rate of diapause completion was considerably higher in the mites exposed to the short-night photoperiods. So, diapausing females appear to be sensitive to photoperiods as different as the two applied by Koveos and colleagues.

In our experiments the sensitivity of diapausing mites to differences in photoperiods —with dark phases that are all shorter than the critical nightlength— appears to be more complex. We did not find any effect of different photoperiods on the mean duration of diapause. We did find, however, a clear effect of nightlength on the amount of variation between individual mites at the same regime. The shorter the nightlength of the diapause termination regime —*i.e.* the closer to the critical nightlength— the more synchronized diapause completion is. If the level of synchronization is taken as a measure of sensitivity of the photoperiodic clock, this result could imply that time measurement is more accurate the closer the nightlength is to the critical nightlength. This hypothesis can be tested by exposing mites to a termination regime with dark phases that are even closer to the critical nightlength. We intend to perform such experiments in the near future.

The results of the present study do not allow a decision on the nature of photoperiodic time measurement in *T. urticae*. In both experiments we found no evidence for an effect of photoperiod of either diapause induction or termination regimes on diapause intensity, expressed as mean diapause duration. This could be interpreted as evidence for qualitative time measurement. However, the clear effect of photoperiod of

the termination regimes on synchronization of the mites demonstrates that quantitative aspects do play a role.

REFERENCES

BECK, S.D., 1989 - Factors influencing the intensity of larval diapause in *Ostrinia nubilalis*. *J. Insect Physiol.* **35(2)**, 75-79.

BELL, R.A. & P.L. ADKISSON, 1964 - Photoperiodic reversibility of diapause induction in an insect. *Science* **144**, 1149-1151.

DANKS, H.V., 1987 - Insect Dormancy: An Ecological Perspective. Biological Survey of Canada, Ottawa.

GOMI, T. & M. TAKEDA, 1992 - A quantitative photoperiod response terminates summer diapause in the tailed zygaenid moth, *Elcysma westwoodii*. *J. Insect Physiol.* **38 (9)**, 665-670.

HARDIE, J., 1990 - The photoperiodic counter, quantitative day-length effects and scotophase timing in the vetch aphid *Megoura viciae*. *J. Insect Physiol.* **36(12)**, 939-949.

HODEK, I., 1983 - Role of environmental factors and endogenous mechanisms in the seasonalilty of reproduction in insects diapausing as adults. *In:* Diapause and Life Cycles Strategies in Insects (V.K. Brown & I. Hodek, eds.), 9-33, Junk, The Hague.

KOVEOS, D.S., A. KROON & A. VEERMAN, 1993 - Geographic variation of diapause intensity in the spider mite *Tetranychus urticae*. *Physiol. Entomol.* **18** (in press).

KROON, A. & A. VEERMAN, 1991 - Quantitative aspects of photoperiodic time measurement in the spider mite *Tetranychus urticae*. *Proc. Exper. & Appl. Entomol.*, N.E.V. Amsterdam **2**, 68-73.

LEES, A.D., 1981 - Action spectra for the photoperiodic control of polymorphism in the aphid *Megoura viciae*. *J. Insect Physiol.* **27**, 761-771.

LEWIS, R.D. & D.S. SAUNDERS, 1987 - A damped circadian oscillator model of an insect photoperiodic clock. I. Description of the model based on a feedback control system. *J. Theor. Biol.* **128**, 47-59.

NUMATA, H. & T. HIDAKA, 1983 - Photoperiodic control of adult diapause in the bean bug, *Riptortus clavatus* Thunberg (Heteroptera; Coreidae). II. Termination of diapause induced under different photoperiods. *Appl. Ent. Zool.* **18(3)**, 439-441.

PITTENDRIGH, C.S., 1981 - Circadian organization and the photoperiodic phenomena. *In:* Biological Clocks in Seasonal Reproductive Cycles (B.K. Follett and D.E. Follett, eds.), 1-35, John Whright, Bristoll.

SAUNDERS, D.S., 1981 - Insect photoperiodism - the clock and the counter: a review. *Physiol. Entomol.* **6**, 99-116.

SPIETH, H.R. & K.P. SAUER, 1991 - Quantitative measurement of photoperiods and its significance for the induction of diapause in *Pieris brassicae* (Lepidoptera; Pieridae). *J. Insect Physiol.* **37(3)**, 231-238.

TAUBER, M.J. & C.A. TAUBER, 1973 - Quantitative response to daylength during diapause in insects. *Nature* **244**, 296-297.

TAUBER, M.J. & C.A. TAUBER, 1976 - Insect seasonalilty: diapause maintenance, termination, and postdiapause development. *Ann. Rev. Entomol.* **21**, 81-107.

TAUBER, M.J., C.A. TAUBER & S. MASAKI, 1986 - Seasonal Adaptations of Insects. Oxford University Press, Oxford.

VAZ NUNES, M., D.S. KOVEOS & A. VEERMAN, 1990 - Geographical variation in photoperiodic induction of diapause in the spider mite (*Tetranychus urticae*): a causal relation between critical nightlength and circadian period? *J. Biol. Rhythms* **5(1)**, 47-57.

VAZ NUNES, M. & A. VEERMAN, 1982 - Photoperiodic time measurement in the spider mite *Tetranychus urticae*: a novel concept. *J. Insect Physiol.* **28**, 1041-1053.

VEERMAN, A., 1977a - Photoperiodic termination of diapause in spider mites. *Nature* **266**, 526-527.

VEERMAN, A., 1977b - Aspects of the induction of diapause in a laboratory strain of the mite *Tetranychus urticae*. *J. Insect Physiol.* **23**, 703-711.

VEERMAN, A., 1985 - Diapause. *In:* Spider Mites: Their Biology, Natural Enemies and Control, Volume 1A (W. Helle and M.W. Sabelis, eds), 279-319, Elsevier, Amsterdam.

ZASLAVSKI, V.A., 1988 - Insect Development: Photoperiodic and Temperature Control. Springer-Verlag, Berlin.

ZASLAVSKI, V.A. & R.B. FOMENKO, 1983 - Quantitative daylength perception in the aphid *Megoura viciae* Buckt. (Homoptera, Aphididae). *Entomol. Obozr.* **62**: 433-441 (in Russian).

GROWTH IN BUMBLEBEE LARVAE: RELATIONS BETWEEN THE AGE OF THE LARVAE, THEIR WEIGHT AND THE AMOUNT OF POLLEN INGESTED BY THEM

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Key words: *Bombus terrestris*, larval development, larval growth

Summary

We measured the weight of worker, male and queen larvae of *Bombus terrestris* of known ages and the amount of pollen they ingested. By this method it was possible to analyse the growth of the three castes. The results show that workers and queens grow in a significantly different way, whereas males and queens grow in a more similar way.

Introduction

Several aspects of the larval development of bumblebees, such as the duration of the larval stage and time of cocoon spinning, larval feeding and growth rates have been studied for some species of *Bombus* (Katayama, 1966; 1973; 1975; Plowright & Jay, 1977; Plowright & Pendrel, 1977; Sutcliffe & Plowright, 1990).

Plowright & Jay (1977) found that food plays a very important role in the size determination of the female larvae of *B. rufocinctus*. They conclude that the adult size is the outcome of differential rates of growth and silk production, which are dependent on the frequency with which the larvae are fed by the workers. Sutcliffe & Plowright (1990), studying *B. terricola* larvae, found that pollen availability may strongly affect their development time. They found that pollen deprivation tends to lengthen the larval stage of the three castes.

These ideas and our information that *B. terrestris* larvae of identical age and weight could have very different amounts of pollen present in their intestine (unpublished data), led us to plan this experiment. By measuring the weight of worker, male and queen larvae of known ages and the amount of pollen they had ingested, we tried to increase our knowledge about larval growth. In particular, we wanted to obtain an answer to the following question: do the three castes have a different kind of growth?

Material and methods

For our experiment we used worker, male and queen larvae from *Bombus terrestris* colonies kept in the laboratory (28°C, 60% RH).

We monitored the egg cups of the second and third broods so that we would know the age of the resulting larvae (in days). Some individuals from several egg cups were collected and weighed (to an accuracy of 0.1 mg).

In the second brood all the eggs are usually diploid, and give rise to female larvae. Therefore, only one larva was taken from each egg cup for the verification of the sex using the method of Duchateau & van Leeuwen (1990). In the third brood, however, the queen may suddenly switch from laying diploid eggs to lay haploid eggs, and both types of eggs can be present within a single egg cup. For that reason, we examined the sex of all the larvae collected. In the majority of cases, we could differentiate the worker larvae from the queen larvae because we followed the development of the larvae which were left in the egg cups. In the cases where we could not distinguish the worker larvae from queen larvae, these larvae were discarded.

The collected larvae were stored in the freezer prior to dissection. So the intestines were removed, macerated and diluted in a sugar-water solution (58%). From a sample of this solution, the number of pollen grains were counted in an haemocytometer, and thereafter the total amount of pollen per larva was estimated.

We analysed 523 individuals: 134 workers (from 5 up to 10 days old); 298 males (5-14 days) and 91 queens (5-15 days, except for 10 days for which unfortunately we could not get the data). We have considered day 5th as the first day of the larval stage, day 0 being the day on which the egg was laid.

Results and Discussion

We found that there is a strong correlation between the weight of the larvae and the amount of pollen they had ingested. The correlation coefficients were: 0.977, 0.926 and 0.945 for workers, males and queens, respectively. This confirms that the weight obtained by the larvae is directly related to the pollen they ingest through their development.

Figures 1 and 2 show the results we obtained for the worker, male and queen larvae with regard to their weight and the amount of pollen they had ingested in relation to age.

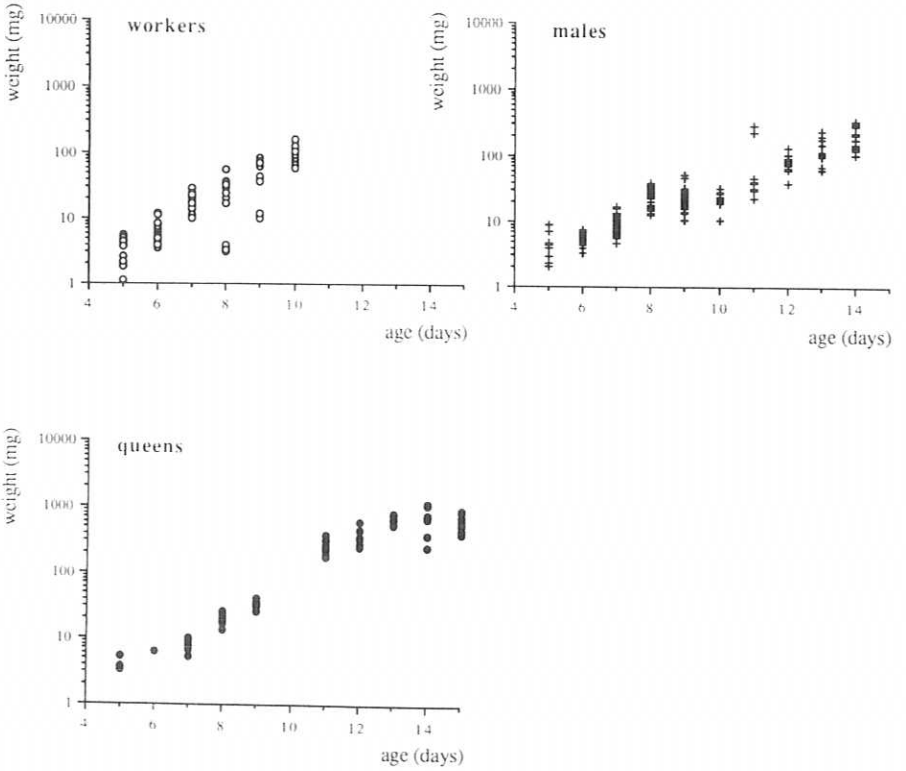

Fig. 1- Increase in weight (mg, on a logarithmic scale) in relation to age (in days) for worker, male and queen larvae.

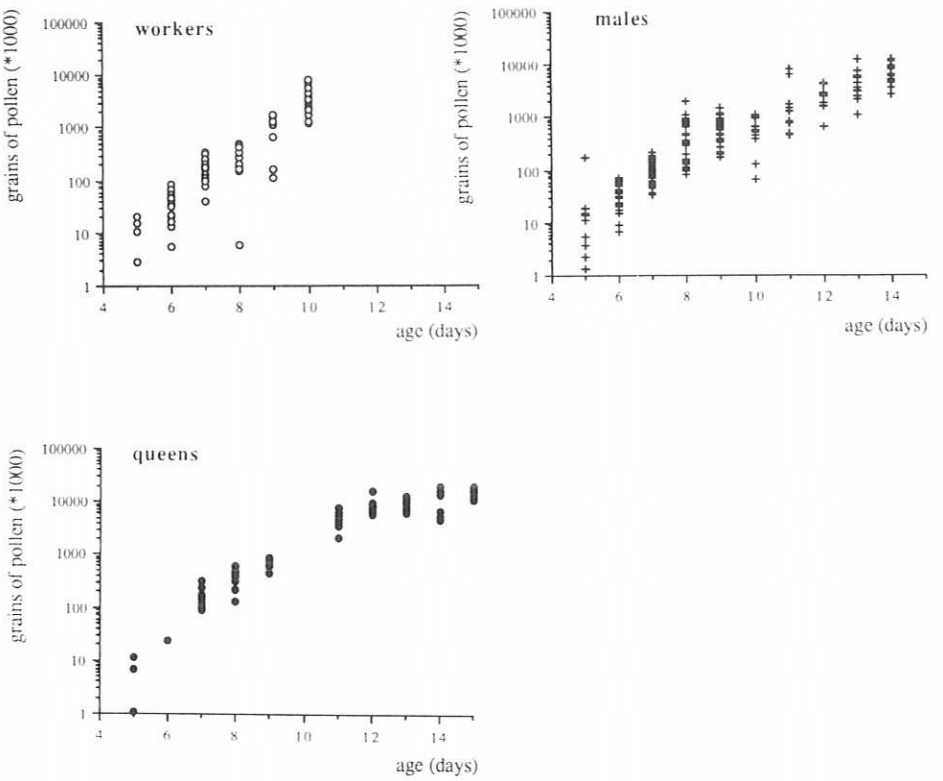

*Fig. 2- Increase in the amount of pollen ingested (number of grains *1000, on a logarithmic scale) in relation to age (in days) for worker, male and queen larvae.*

In general, workers reach higher values faster than males and queens. For example, on the 9th day the maximum weight of the workers was approximately 80 mg, whereas males had around 50 mg and queens only about 40 mg. With regard to the amount of pollen, workers had already ingested almost 2 million grains, whereas males had ingested nearly 1.5 million and queens only 1 million grains. The body weight and the amount of pollen ingested indicate that queens grow much more slowly than workers. However, we also found that workers show lower final maximum values for body weight (around 170 mg) and amount of pollen consumed (around 8.5 million grains) than males and queens. The corresponding figures for the queens were: 1200 mg of weight and 22 million pollen grains. In queens apparently the slower growth rate is compensated by a longer development period.

To find out whether the growth of the three groups of larvae was significantly different we applied Multiple Regression Analysis (Sokal & Rohlf, 1981), taking weight (logarithmically transformed) as a dependent variable (y) and age, larvae and the logarithm of pollen as independent variables (x). The critical values for significance were: $p < 0.05$, * = significant; $p < 0.005$, ** = highly significant (table 1).

We made two comparisons: a) the three groups (workers, males, queens); b) the female larvae only (i. e. workers and queens).

Table 1- Multiple Regression Analysis for worker, male and queen larvae, with the single variables (larva, age and pollen) and the interactions (larva/age and larva/pollen).
(Legend: w= workers, m= males, q= queens, qw= queens compared to workers, qm= queens compared to males, n.s.= not significant)

kind of comparison	w/m/q	w/q
independent variable	(r^2=0.947)	(r^2=0.962)
qw	**	**
qm	**	
age	**	**
pollen	**	**
qw/age	**	**
qw/pollen	**	**
qm/age	*	
qm/pollen	n.s.	

With regard to the single variables, we come to the following conclusions:
-when compared, queens and workers are always very different, with or without the presence of males, indicating that the males do not affect the comparison;
-queens and males are also very different;
-age and pollen are important factors in the increase in weight.

Furthermore, with regard to the interactions between the variables, we find that:
- queens and workers increase very differently in weight in relation their age and the amount the pollen ingested;
- queens and males increase differently (but not very significantly) in weight in relation to their age, but they do not differ at all in relation to the amount of pollen ingested.

However, our results do not agree with the results found for other species. Katayama (1975) did not find any significant difference in the growth curves for the three castes of *B. hypocrita*, although some of his male and queen batches did develop slowly in the early stages. Plowright & Pendrel (1977) showed that in *B. terricola* the growth of queens also did not differ from that of workers. They suggested that the queen morphogenesis was probably characterized mainly by a longer larval stage, although the caste could be determined earlier. However, in our view, at least for *B. terrestris*, this cannot be the main factor determining the queens' development. Our results, on the other hand, show that queens and workers have significantly different growth rates: the workers grow faster than queens. In addition, it is well known that those worker larvae that take even longer to develop (up to 12 or 13 days), never attain the same size as the queen larvae. Therefore, the different rate of growth must play a more important role in the development of workers and queens than the different lengths of the larval stage.

Acknowledgements

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References

DUCHATEAU, M. J. & VAN LEEUWEN, P., 1990. Early sex determination in larvae of *Bombus terrestris*. *Insectes Sociaux*, **37**(3): 232-235.

KATAYAMA, E., 1966. Studies on the development of the broods of *Bombus diversus* Smith (Hymenoptera, Apidae) II. Brood development and feeding habits. *Kontyû*, **34**(1): 8-17.

KATAYAMA, E., 1973. Observations on the brood development in *Bombus ignitus* (Hymenoptera, Apidae) II. Brood development and feeding habits. *Kontyû*, **41**(2): 203-216.

KATAYAMA, E., 1975. Egg-laying habits and brood development in *Bombus hypocrita* (Hymenoptera, Apidae) II. Brood development and feeding habits. *Kontyû*, **43**(4): 478-496.

PLOWRIGHT, R. C. & JAY, S.C., 1977. On the size determination of bumble bee castes (Hymenoptera: Apidae). *Can. J. Zool.*, **55**: 1133-1138.

PLOWRIGHT, R. C. & PENDREL, B.A., 1977. Larval growth in bumble bees (Hymenoptera, Apidae). *Can. Ent.*, **109**: 967-973.

SOKAL, R.R. & ROHLF, F.J., 1981. *Biometry* (2nd ed.). W. H. Freeman and Co. San Francisco. 859 pp.

SUTCLIFFE, G. H. & PLOWRIGHT, R. C., 1990. The effects of pollen availability on development time in the bumble bee *Bombus terricola* K. (Hymenoptera: Apidae). *Can. J. Zool.*, **68:** 1120-1123.

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EFFECTS OF RABBIT AND CATTLE GRAZING ON GRASSHOPPERS (Orthoptera: Acrididae) OF RIVER DUNES

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Key words: Acrididae, model, grazing, vegetation structure, nature management

Summary

We test some implications of a conceptual model describing the effects of grazing intensity and soil nutrient availability on field layer insects with grasshopper samples from *Carex arenaria* dominated river dunes. The results sustain the hypothesis that removal of vegetation by grazers has a negative effect on a number of grasshopper species. Model and results lead to the provisional recommendation to exclude nutrient poor, warm and dry biotopes from grazing.

INTRODUCTION

Introduction of cattle is applied more and more for managing nature reserves. The manager's objective is to bring about more variation in vegetation and soil, and the removal of brushwood. Despite the widespread application of grazing as a nature management measure little is known on the effects on the invertebrate fauna. Comparative faunistic surveys in grazed and ungrazed areas indicate decrease of species diversity (Siepel et al., 1989), loss of Carabid species (Oova & Van Steenis, 1988), and a reduction of the mean individual weight (Siepel, 1990). Furthermore, Verstegen et al. (1992) observed the absence of the typical xerothermic heathland fauna on grazed heathlands. Exceptions to these negative effects are the higher species number and overall grasshopper abundance in grazed grass dominated heathland (Van Wingerden et al. 1991b); this difference, however, is predominantly caused by higher numbers of only one species, *Stenobothrus stigmaticus* (Rambur).

As faunistic research on the effects of light grazing of vaste areas in particular is extremely laborious and long-lasting, we have chosen to contribute to the grazing debate by broaching the question in which way grazing influences insects at the population level.

Sampling results concerning grasshoppers in nutrient rich heath- and grasslands led to the hypothesis that the relationship between the density of all species together and grazing intensity can be described best by a Gauss curve (Van Wingerden et al., 1991b): under conditions of nutrient richness and very light grazing, the grasshopper density primarily increases, but under conditions of heavy grazing the density decreases (Fig 1., curve I). This model may also go for the density of certain separate species, as suggested by fitting density data of *S. stigmaticus* from different samplings to a quadratic model (Fig. 2).

An alarming implication of this model is that in (parts of) areas where conditions for certain species are optimal - or suboptimal at the

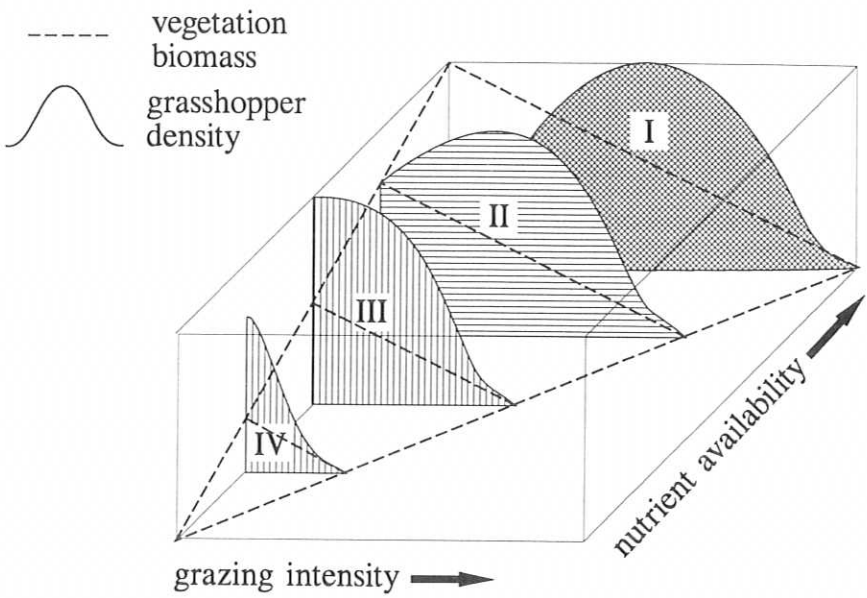

Figure 1. Conceptual model describing the relationship of grasshopper density and vegetation biomass with grazing intensity and soil nutrient availability. Shape Gauss-curve from Ernst & Van Andel, 1985.

maximum tail of the model -, grazing can only cause densities to fall (Van Wingerden et al., 1991b; Fig. 1, curves III, IV). For grasshoppers such optimal conditions are met with in rather sparse vegetations which usually occur on soils which are poor in nutrients. Therefore, we extended our conceptual model by adding the predictor soil nutrient availability to it (Fig. 1). It is the main objective of the present study to test the forementioned implication. Therefore, the grasshopper fauna of *Carex arenaria* dominated river dunes seemed to be appropriate. Its vegetation has a open structure, even under conditions without grazing, allowing the light radiation heat to be absorbed by the topsoil. Moreover, it has a sandy soil which does not remain wet very long, and consequently warms up quickly. These conditions favour egg development of some species, thermophilous species in particular (Van Wingerden et al., 1991a), whereas the vegetation offers enough shelter for the survival of nymphs and imagines (Van Wingerden et al., 1991b). We expected this biotope - under conditions without grazing - to be more or less optimal for some grasshopper species, and their reaction to grazing to be described best by the curves III and IV from our conceptual model (Fig. 1).

STUDY-AREAS AND METHODS

- study areas

We sampled in three areas at the borders of the river Overijsselse Vecht, viz. Junnerkoeland (JK), Junnerkoeland-Oost (JK-O) and Oud-Bergentheim (OB). In JK (grazed part measures 39.5 ha) summer season grazing has been applied with 40 - 45 head of young cattle since

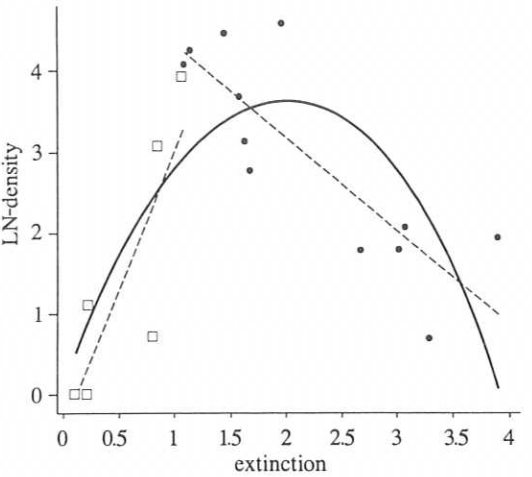

Figure 2. Results of fitting data on density (N/are) of Stenobothrus stigmaticus (Rambur) and mean light radiation extinction from two data sets to a quadratic model. □ = Junnerkoeland 1988; ● = Deschampsia flexuosa dominated heathland in the surroundings of Arnhem 1989. Data from Van Wingerden et al. (1991b).

1967. In addition, there lives a large rabbit population which is unevenly distributed, and produces large density fluctuations. Here we sampled all *Carex arenaria* plots in sofar their surface level being higher than 5.50 m above N.A.P., their area being larger than 400 m2 and they were uncovered by brushwood or trees as well; in this way we selected nine plots for sampling one of which being situated inside the exclosure in which cattle grazing is prevented.

In JK-0 (grazed part measures 5.5 ha) summer season grazing has been applied with 6 - 7 head of young cattle since 1980. Here four plots were sampled, two of which being grazed very lightly, and two others being grazed and trampled by rabbits and cattle, respectively, more severely (Tab. 1). Primarily JK and JK-0 were grazed by separate herds, but since 1989 by one herd of about 40 head of young cattle.

OB (1 ha) has not been grazed by cattle. Fertilizing and haying have been applied for the last time in 1979, and ever since management measures were not performed anymore. Here we sampled three plots with differing levels of rabbit grazing (Tab. 1).

- sampling

We sampled grasshoppers from 16 sample units of 1 m^2 from plots of 400 m^2 (20 x 20 or 10 x 40 m) each, during the last decade of August 1991. We simultaneously placed two gauze biocoenometers with a bottom surface of 0.5 m^2 each very quickly, and adjacently on random selected points. We caught the grasshoppers from the inside of the biocoenometer with a motor driven aspirator. After sampling a plot we identified the grasshoppers in the field and released them, except the individuals from the *C. biguttulus/C. brunneus/C. mollis* group which we kept for further examination. Those were identified by means of a first selection on the basis of the male song observed in the plot, and secondly by means of measuring wing lengths and costal area widths (Perdeck, 1957). We attributed the females from these group among the species according to the male species ratio.

We calculated light radiation extinction on 40 points along a transect of 20 m in the middle of the plot, as a measure for the aboveground vegetation, from simultaneous measurements at the soil surface as well as over the vegetation with two optical line sensors of 30 cm each. We counted rabbit droppings on a surface area of 10 m^2 in

Table 1. Study areas, plots, environmental variables and grasshopper number per 16 m². JK = Junnerkoeland (plots A - H, Exclosure); JK-O = Junnerkoeland-Oost (plots K - N); OB = Oud-Bergentheim (plots P, Q, R); CD = number of Cattle Dung droppings per 400 m²; RD = number of Rabbit Droppings per 10 m²; Ext = light radiation Extinction by the vegetation at the soil surface (mean from 40 points); Stig = Stenobothrus stigmaticus (Rambur); Para = Chorthippus parallelus (Zetterstedt); Bigu = C. biguttulus (L.); Omo = Omocestus viridulus (L.); Myrm = Myrmeleotettix maculatus (Thunberg); Moll = C. mollis (Charpentier); Brun = C. brunneus (Thunberg).

JK	CD	RD	Ext	Stig	Para	Bigu	Omo	Myrm	Moll	Brun
A	3	878	0.02	0	0	0	0	0	0	0
B	53	67	0.89	27	3	5	0	0	0	0
C	83	1105	0.50	8	8	1	0	0	0	0
D	4	78	1.27	45	6	2	2	0	0	1
E	3	460	0.45	17	0	0	0	0	0	0
F	11	717	0.87	71	2	0	0	0	0	0
G	267	337	0.56	5	1	16	0	22	0	0
H	21	829	0.78	27	8	20	0	0	0	0
EXCL.	0	503	2.25	38	6	28	8	0	0	6
JK-O										
K	8	9	2.26	49	48	13	11	0	76	0
L	8	10	1.25	58	38	14	6	1	66	0
M	46	205	0.37	23	1	0	0	1	33	0
N	37	406	0.73	17	0	0	0	0	43	0
OB										
P	0	224	3.56	29	31	58	1	0	0	0
Q	0	1628	0.11	5	0	2	0	0	0	0
R	0	860	0.68	11	15	20	0	0	0	0

the middle of each plot as a measure for the grazing intensity of rabbits, and the number of cow droppings on each plot as a measure for the grazing and trampling intensity of cattle.

- analysis

We did regression analysis with plot as predictor and grasshopper number per sample unit of 1 m² as response variable in order to test for plot differences.

As we assumed grazing intensity to influence grasshoppers through its effect on vegetation biomass, we decided to analyse any relationships between grazing intensity and vegetation biomass, vegetation biomass and grasshopper density, and directly, grazing intensity and grasshopper density. Therefore, we did regression analysis on the plot level with mean light radiation extinction (which we assumed to be strongly related to vegetation biomass) as predictor and grasshopper mean density as response variable; furthermore, with rabbit dropping number, cow dropping number and (whether or not) cattle grazing (was applied) as predictors and light radiation extinction as response variable; finally, with the same predictors, but with grasshopper plot density as response variable.

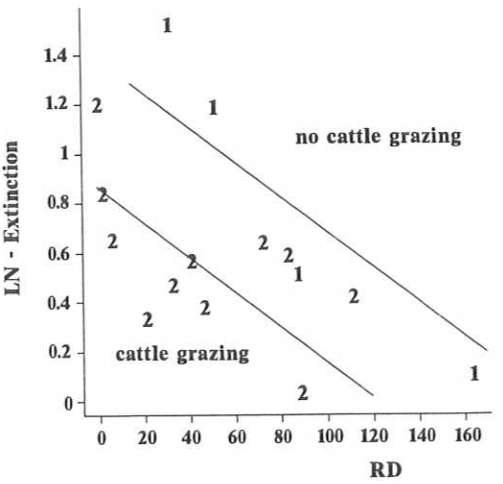

Figure 3. Results of regression analysis of light radiation extinction on rabbit dropping numbers (RD, N/m²) and cattle grazing, on all plots. 1 = no cattle grazing; 2 = cattle grazing

RESULTS

- differences between plots

Variation in the grasshopper LOG_numbers from the sample units is larger among plots than within, for all seven species separately, and for all species together (linear regression analysis; F-test, two-tailed; $P < 0.001$). This leads to rejection of the hypothesis that no differences exist between the plots.

- effect of grazing intensity on vegetation biomass

LOG_light radiation extinction (as measure for aboveground vegetation biomass) is negatively correlated with the combination of rabbit dropping number and (whether or not) cattle grazing ($P < 0.001$, Table 2, Figure 4). The addition of cattle grazing should be interpreted with care as it is partly confounded with area effects. There is also a negative correlation with rabbit dropping number only ($P < 0.05$). No correlation was found with cow dropping number.

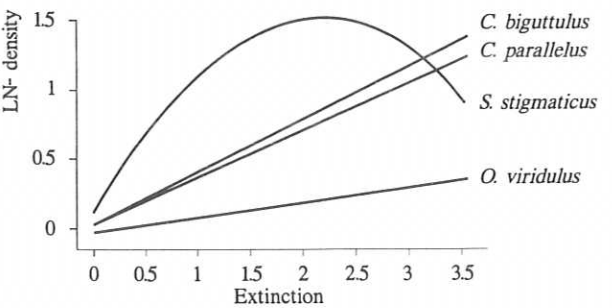

Figure 4. Results of regression analysis of plot densities (N/m²) of four grasshopper species, on mean light radiation extinction per plot, on all plots. Curves are fitted values. See for regression equations Table 2.

- effect of vegetation biomass on grasshopper density

Plot LOG_density of *C. biguttulus* ($P < 0.001$), *C. parallelus* ($P < 0.01$) and *O. viridulus* ($P < 0.01$) are positively correlated to (LOG_)light radiation extinction. For *S. stigmaticus* and for all species together ($P < 0.001$) this relationship was described best by a quadratic curve: at low extinction values density increases, but at extinction values higher than 2 density decreases (Table 2, Figure 4). For the density of all species together minus *S. stigmaticus*, however, addition of a quadratic term for extinction does not give better fit than with linear regression ($P < 0.01$; Figure 6). The percentages of variance accounted for are rather high, for all species even 71%, indicating an important role of vegetation biomass in the determination

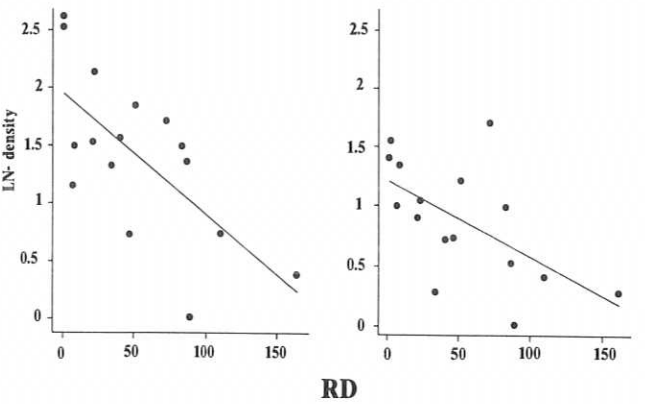

Figure 5. Results of regression analysis of plot densities (N/m^2) of all species together (left) and of S. stigmaticus (right) on rabbit dropping numbers (RD, N/m^2) per plot, on all plots. See for regression equations Table 2.

Table 2. Results of regression analyses between parameters for grazing intensity and grasshopper densities. All tests are F-tests. A = All grasshopper species together; B = C. biguttulus; S = S. stigmaticus; Pa = C. parallelus; O = Omocestus viridulus; A-S = All species minus S. stigmaticus; CG = Cattle Grazing; RD = number of Rabbit Droppings.10m^{-2}; Ext = light radiation Extinction at the soil surface; percentage is percentage of variance accounted for.

LN_Ext = 1.4 - 7.0*10^{-4}RD - 5.3*10^{-1}CG	[P < 0.001]	58%
LN_Ext = 8.9*10^{-1} - 5.1*10^{-4}RD	[P < 0.05]	28%
LN_density B = 2.0*10^{-2} + 3.8*10^{-1}Ext	[P < 0.001]	56%
LN_density S = 8.4*10^{-2} + 1.3Ext - 3.0*10^{-1}Ext2	[P < 0.001]	61%
LN_density Pa = 3.2*10^{-2} + 3.4*10^{-1}Ext	[P < 0.01]	44%
LN_density O = - 8.2*10^{-2} + 2.7*10^{-1}LN_Ext	[P < 0.01]	36%
LN_density A = 2.7*10^{-1} + 1.7Ext - 3.5*10^{-1}Ext2	[P < 0.001]	71%
LN_density S = 1.2 - 6.3*10^{-4}RD	[P < 0.05]	29%
LN_density A = 2.0 - 10.7*10^{-4}RD	[P < 0.01]	43%
LN_density A-S = 1.4 - 9.3*10^{-4}RD	[P < 0.05]	26%
LN_density B = 1.7 - 1.3CG	[P < 0.05]	33%
LN_density A-S = 3.9*10^{-1} + 5.4*10^{-1}Ext	[P < 0.01]	40%

of density (Table 2). No correlation was found for *C. brunneus*, *M. maculatus* and *C. mollis*.

- **effect of grazing intensity on grasshopper density**

Plot LOG_density of *S. stigmaticus* ($P < 0.05$) is negatively correlated with rabbit dropping number (Table 2, Figure 5). This also goes for LOG_density of all species together ($P < 0.01$), and of all species minus *S. stigmaticus* ($P < 0.05$, Table 2). Plot LOG_density of *C. biguttulus* is negatively correlated to cattle grazing ($P < 0.05$, Table 2). The latter correlation should be interpreted with care as cattle grazing is partly confounded with area effects. No correlation was found between these predictors and other species, nor with cow dropping number as predictor.

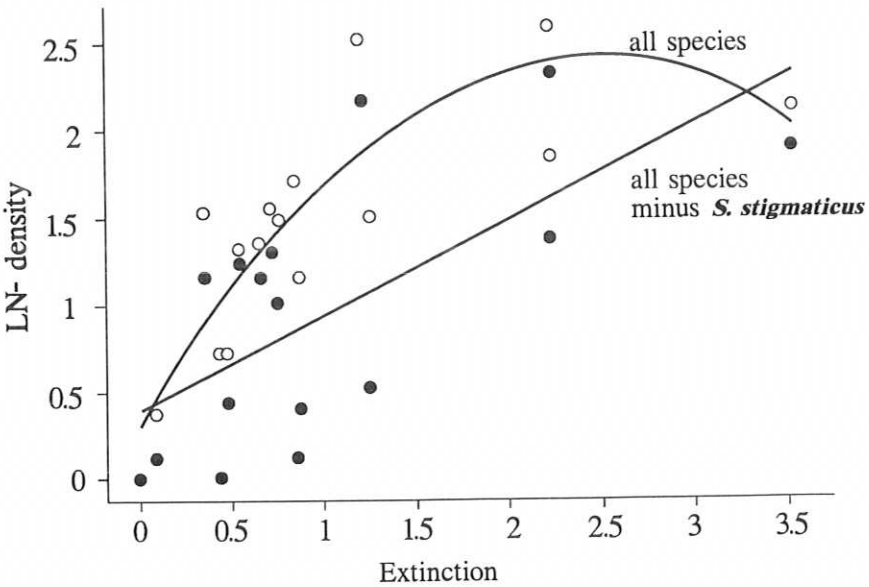

Figure 6. Results of regression analysis of plot densities (N/m²) of all species together (o) and idem, minus S. stigmaticus (●) on light radiation extinction per plot, on all plots. See for regression equations Table 2.

DISCUSSION

The negative correlation between aboveground vegetation biomass and the combination of rabbit dropping number and cattle grazing (Figure 3) agrees with the expectations from our conceptual model (Figure 1, nutrient levels III, IV). The positive correlation between the density of three species (*C. biguttulus, C. parallelus, O. viridulus*) each and vegetation biomass (but now used as a measure for grazing intensity) also agrees with our model (Figure 1, curves III, IV). For *S. stigmaticus* curve II is a better model than curve III. Very light grazing may have a positive effect on the density of this species, whereas greater grazing intensity may act negatively. The exceptional position of this species agrees with its positive reaction to intensive

grazing on grass dominated heathlands, whereas in other species the density did not increase or hardly (Van Wingerden et al., 1991b). This species apparently tolerates a rather heavy grazing pressure, whereas other grasshopper species do not. Because of the dominance of *S. stigmaticus* in the grasshopper samples (Table 1) the best fitting model of the density of all species dependent on vegetation biomass has consequently become a quadratic one (Figure 3). The density of all species except *S. stigmaticus* together fits better to a linear model (Figure 6). The latter correlation as well as the forementioned ones between three species each and vegetation biomass lead to the hypothesis that removal of vegetation biomass by grazing eliminates individuals of three grasshopper species from river dunes, as is being demonstrated by their absence on the plots A, E, and N (Table 1). The absence of correlation between the density of *C. brunneus*, *M. maculatus* and *C. mollis* and vegetation biomass may be accounted for by differences between plots or areas other than vegetation biomass and grazing intensity. *C. mollis* lives in high densities in JK-O but is nearly absent in JK and OB. To explain this difference we formulated the ad hoc-hypothesis that JK-O is warmer than the other ones, as a consequence of its relief and sheltered position. This warmer mesoclimate may favour *C. mollis* which needs more heat for its egg development than other soil ovipositing species, such as *C. biguttulus*, *C. parallelus*, *C. brunneus* (Van Wingerden et al., 1991a).

Consequently we expected also a direct relationship between grasshopper density and our parameters for rabbit and cattle grazing (and trampling) intensity. We found, however, a negative correlation between plot density and rabbit dropping number for only one species, viz. *S. stigmaticus*. Here a quadratic curve did not fit better than a linear one, like in the regression with vegetation biomass as predictor. Furthermore, we found negative correlations for all species together and idem, minus *S. stigmaticus*. The lack of correlation for *C. parallelus* and *O. viridulus* with rabbit dropping number, the lack of any correlation with cow dropping number as well as the lower levels of significance as compared to the correlations with vegetation biomass (Table 2) lead to the ad-hoc hypothesis that we have measured rabbit and cattle dropping number during the wrong time interval, e.g. that rabbit and cattle grazing influence vegetation biomass predominantly earlier in the season or during preceding years already. This influence may not be reflected by our measurements on cattle and rabbit dropping numbers during the sampling period. A different explanation for the lack of fit may be the dissimilarity between the area in which rabbit droppings were counted, and the grasshopper sample units within each plot.

Both the negative correlation between density of all species (minus *S. stigmaticus)* and rabbit dropping number, the one between density of *C. biguttulus* and cattle grazing and the positive correlation between density of three species and vegetation biomass sustain the from the conceptual model derived implication that on nutrient poor soils even light grazing has already a negative effect on density, at least for three species. From the differences with respect to vegetation biomass between *C. biguttulus*, *C. parallelus* and *O. viridulus* on the one hand and *S. stigmaticus* on the other hand we conclude that the optimum for grazing intensity differs among species. Furthermore the high percentages accounted for of light radiation

extinction indicate a major role of vegetation biomass in the determination of the density of grasshopper populations under the influence of grazing, as was indicated by Van Wingerden et al. (1991a, 1991b, 1992a, 1992b) for other biotopes. The analysis results do not lead to rejection of our conceptual model, but further evidence has to be produced before common management rules can be obtained from it. Firstly, the role of both predictors - soil nutrient availability and grazing intensity - has to be studied in one experimental design. Secondly, the effect of grazing through heterogeneity has to be covered. Furthermore, the other effects of grazing such as trampling, fertilizing and perturbation must be included and other animal groups groups as well.

The grasshopper species dealt with in the present study are neither rare in our country, nor threatened. Moreover, they also occur in the lower and grass dominated parts of the study areas. By presenting and testing our conceptual model, however, we make an example of what grazing may bring about to rare or threatened animal species being confined to biotopes being similar to river dunes because they need warm and dry conditions and shelter as well, such as the Wartbiter (*Decticus verrucivorus* (L.)), the Field Cricket (*Gryllus campestris* L.), the Sand Lizard (*Lacerta agilis* (L.)) and the spider *Eresus niger* (Petagna). For this moment we recommend the nature management organisations to be restrictive at the allowance of grazing in such structure rich and xerothermic biotopes. This advice may even go for all biotopes which fulfill the requirements of nature conservation priority species optimally. Here grazing may threaten the persistence of populations. Very often it is argued that this threat is negligible if the grazed area is very large. The assumption behind this thought is that in very large areas part of the valuable sites will remain ungrazed, as the grazers will not visit all of them. This assumption may be doubted for in the case of river dunes. Firstly, because rabbits aggregate around these dunes. Secondly, because in addition cows prefer to ruminate, rest and graze on and around these dunes, which offer shelter, and have a relatively dry and - on sun-oriented slopes - warm soil as compared to lower parts of river border grasslands. Especially, when site preference of grazers and nature conservation priority species coincide, grazing may threaten the persistence of populations of the latter. Therefore it might be wise to exclude nutrient poor, warm and dry biotopes, such as river dunes (partly) from grazing until more knowledge on interactions between grazers and xerothermic animal species has been produced.

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REFERENCES

ERNST, W.H.O. AND ANDEL, J. VAN, 1985 - Adaptaties, voornamelijk van oecofysiologische aard. In: *Inleiding tot de oecologie* (K. Bakker ed.): 70-100. Bohn, Scheltema & Holkema, Utrecht.

OVAA, A.H. AND STEENIS, M.J. VAN, 1988 - Heidebeheer en Heidefauna op de Wolfhezerheide. *Verslag Natuurbeheer Landbouwuniversiteit Wageningen*, **969**, 1-53.

PERDECK, A.C., 1957 - *The isolating value of specific song patterns in two sibling species of grasshoppers (Chorthippus brunneus Thunb. and C. biguttulus L.)*. 1-75. Brill, Leiden.

SIEPEL, H., MEIJER, J., MABELIS, A.A., AND BOER, M.H. DEN, 1989 - A tool to assess the influence of management practices on grassland surface macrofaunas. *J. appl. Ent.* **108**, 271-290.

SIEPEL, H., 1991 - Nature restoration and the role of the mesofauna in decomposition of organic matter. *Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam*, **2**, 22-27.

VERSTEGEN, M.A.J.M., SIEPEL, H., STUMPEL, A.H.P. AND WIJNHOVEN, H.A.H., 1992 - Heide en heidefauna: indicaties voor het beheer. *RIN-rapport* **92/26**, *Arnhem: DLO Institute for Forestry and Nature Research*, 1-126.

WINGERDEN, W.K.R.E. VAN, MUSTERS, J.C.M. AND MAASKAMP, F.I.M., 1991a - The influence of temperature on the duration of egg development in West European grasshoppers (Orthoptera: Acrididae). *Oecologia* **87**, 417-423.

WINGERDEN, W.K.R.E. VAN, MUSTERS, J.C.M., KLEUKERS, R.M.J.C., BONGERS, W. AND BIEZEN, J.B., 1991b - The influence of cattle grazing intensity on grasshopper abundance (Orthoptera: Acrididae). *Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam*, **2**, 28-34.

WINGERDEN, W.K.R.E. VAN, KREVELD, A.R. VAN AND BONGERS, W., 1992a - Analysis of species composition and abundance of grasshoppers (Orth., Acrididae) in natural and fertilized grasslands. *J. Appl. Ent.* **113**, 138-152.

WINGERDEN, W.K.R.E. VAN, MUSTERS, J.C.M., CANNEMEIJER, F. AND BONGERS, W., 1992b - Simulation of hatching dates in three *Chorthippus* species (Orthoptera: Acrididae) in unfertilized and lightly fertilized grasslands. *Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam*, **3**, 86-93.

THE RELATIVE IMPORTANCE OF SYRPHIDS AND BUMBLEBEES AS POLLINATORS OF THREE PLANT SPECIES

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Key words bumblebees, syrphids, foraging speed, pollen deposition, seed set

Summary

The pollination value of insect visitors to three plant species, *Phyteuma nigrum* (Campanulaceae), *Succisa pratensis* and *Scabiosa columbaria* (both Dipsacaceae), was determined. The most numerous insects on all three species were flies, especially syrphids. Other visitors were bees, especially bumblebees, butterflies and a few beetles. Visitation time of syrphids on *P. nigrum* was much longer than that of bumblebees. However, seed set after syrphid pollination in cages (0.5 and 2 flies per inflorescence) was very low compared with open (bumblebees plus syrphids) pollination. Bumblebees visited 2-3 times more flower heads of *S. pratensis* and *S. columbaria* per minute than did syrphids. On *S. pratensis* and *S. columbaria* bumblebees deposited more pollen grains per stigma (8.1 and 6.1 respectively for the two plant species) than did syrphids (5.4 and 3.7). Although fewer in number, bumblebees are the primary pollinators of *P. nigrum* and, under bad weather conditions, the only pollinators of *S. pratensis* and *S. columbaria*. Nevertheless, if larger syrphids, such as *Eristalis*, *Helophilus* and *Syrphus* s.l. are present, they are capable of pollinating the latter two plant species.

INTRODUCTION

Many plant taxa are visited by several animal taxa. These taxa may differ in number of individuals and such foraging characteristics as flower constancy, pollen carrying capacity, or number of flowers visited per time. Pollination efficiency may differ between various groups of visitors to the same plant species (Spears 1983, Sugden 1983, Waser and Price 1990, Dieringer 1992). Sometimes visitors are not pollinators at all due to their behaviour (nectar-thieves or pollen thieves (Inouye 1980)), due the absence of conspecific pollen on the bodies, or due to to their failure to deposit compatible pollen on stigmas. Thus the pollination value of visitors has both quantitative and qualitative components (Herrera 1987).

This paper presents data on the visit frequency (quantitative component) of syrphids and bumblebees and their pollination efficiency (qualitative component) on three plant species: *Phyteuma nigrum* (Campanulaceae), *Succisa pratensis* and *Scabiosa columbaria* (both Dipsacaceae). These species have a protruding accessible stigma, open pollen presentation, and short tubular flowers with nectar.

MATERIALS AND METHODS

Phyteuma nigrum

Phyteuma nigrum (Dark Rampion) is a perennial outbreeding species that flowers May-June, in the Netherlands occurring mainly in nature reserves. The flowers are protandrous with secondary pollen presentation. Nectar is present mainly in the male phase flowers. The frequency of flower visiting insects was scored in two transects of 15 m^2 in the nature reserve Stroomdallandschap Drentse Aa, 30 km south of Groningen (the Netherlands), 16-20 times per day from 0800 to 1800 hours on three days during early, peak and late flowering in 1989, 1991 and 1992. The number of inflorescences was counted every observation day.

The foraging time spent per inflorescence by bumblebees and syrphids visiting *P. nigrum* was measured by following individual insects and measuring the time spent per open flowering inflorescence (1989).

Pollination efficiency of syrphids was measured during 1992 in an experiment with caged flowers. A known number of syrphids (0.5 and 2 flies per inflorescence) was introduced into cages with known numbers of inflorescences, one inflorescence , promoting optimal outcrossing (*P. nigrum* is self-incompatible but outcrossing distance has no effect on seed set, unpubl. data). Before the introduction of the syrphids the virgin flowers were marked. Twenty days later the marked capsules were collected and the number of developed seeds was counted. These seed sets were compared with seed sets of open pollinated (bumblebee and syrphid visited flowers) and of permanently caged, insect free flowers.

Succisa pratensis

Succisa pratensis (Devilsbit Scabious), a perennial herb, occurs occasionally in unfertilized hay fields and along road sides in the Netherlands. The small tubular flowers, found August-October, are arranged in heads (about 60 per head). Each is first male for 8-9 days with protruding anthers, finally becoming female with protruding stigmas for one day. Nectar is present both in male and female flowers. The frequency of flower visiting insects was scored in three populations once a week between 1000 and 1600 hours, during the main period of flowering. The populations were Kappersbulten (1990), Assen (1990), and Geelbroek (1992).

Foraging speed, number of flower heads visited per minute, was measured by following individual insects during their foraging trip (Kappersbulten 1990).

Pollen deposition by bumblebees and syrphids on stigmas of virgin female flower heads was determined in the field by counting grains using a 20x magnification (Geelbroek 1992).

Scabiosa columbaria

Scabiosa columbaria (Small Scabious) is a perennial, outbreeding species, rare in the Netherlands, occurring in dry, grassy places on calcareous soils. The blue-violet flowers, occurring July-September, are arranged in heads (about 70 per head). Each is first male for several days, then female. Nectar is present in both male and female flowers. The frequency of flower visiting insects was scored in two permanent plots in an experimental garden (Haren) once a week during the entire flower season of 1991 and once in a natural population in the middle of the Netherlands (Olst 1991).

Foraging speed, number of flower heads visited per minute, and pollen deposition were measured in the same way as for *S. pratensis* (Haren 1991).

RESULTS

Phyteuma nigrum

In all three observation years bumblebees (*Bombus pascuorum* (queens), as well as workers of *B. pratorum* and *B. jonellus*) and syrphid flies (mainly *Rhingia campestris)* were principal visitors of *P. nigrum*. Occasionally a small bee (*Lasioglossum morio*) was observed and other fly species and butterflies were infrequent. In 1992, large numbers of *Apis mellifera* were visitors. In all three years, however, syrphids were the most numerous visitors, eating pollen directly from the style, pushing down the corolla lobes with their front legs and thus reaching fresh pollen, eating pollen directly from the stigma, or eating pollen cleaned from their body. Bumblebees also collected pollen by pushing down the corolla lobes. During visits of *B. pascuorum* this was done rather passively during nectar collecting but the workers of the short-tongued bumblebees activily used their legs to push down the corolla lobes while supporting themselves on the style. Nectar was collected by introducing the tongue between the corolla lobes into the nectary.

The foraging time per inflorescence was much longer for syrphids (87.9 $\pm$ 13.1 secs, mean $\pm$ SE for *R. campestris*) than for bumblebees (15.0 $\pm$ 3.2 secs for *B. pratorum* and 21.9 $\pm$ 11.0 secs for *B. jonellus*).

Seed set of flowers pollinated by syrphids, bumblebees and syrphids, and of flowers left without insects differed significantly (Mann-Whitney, for all combinations $p < 0.001$). Permanently caged flowers hardly set any seeds, flowers with 0.5 syrphids per inflorescence set about 2 seeds per flower, with 2 syrphids per inflorescence about 4 seeds per flower. Flowers visited by both syrphids and bumblebees set about 22 seeds per flower.

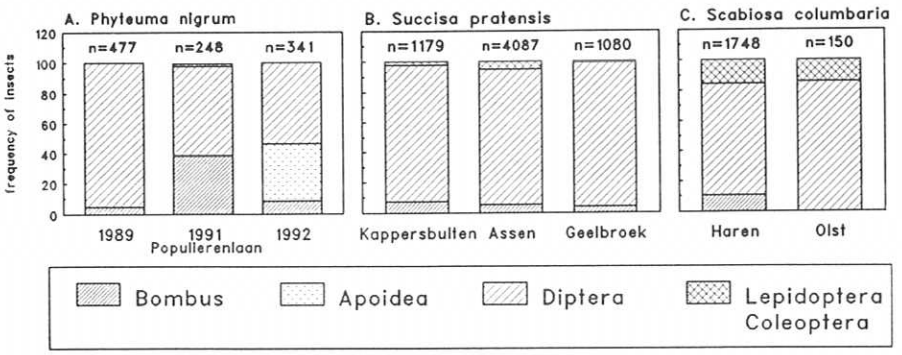

Figure 1: Frequency of total flower visiting insects during the season made by different groups to three plant species

Succisa pratensis

In all populations of *S. pratensis,* the most numerous visitors were flies, especially various syrphid species. In population Kappersbulten the small fly *Siphona geniculatus* (Tachinidae) also became very numerous late in the season. Butterflies were observed early in the season during good weather. Bumblebees, mainly *B. pascuorum,* were observed throughout the flowering season but their numbers were low. Insects visited *S. pratensis* for pollen and or/nectar. Syrphids consumed pollen directly from the anthers, from the stigmas, or from their bodies after cleaning.

The foraging speed of syrphids and bumblebees differed significantly: syrphids visited 2.1 flower heads per minute and bumblebees 4.8 (Table 1). Syrphids rested for long periods between their flower visits (not included in the foraging speed) whereas bumblebees continued foraging throughout the day.

Pollen deposition of syrphids and bumblebees differed significantly. Syrphids (*Eristalis tenax*) deposited 5.4 and bumblebees 8.1 pollen grains per stigma (Table 1).

	Syrphids		Bumblebees		
Succisa pratensis	n		n		statistics
heads per minute	11	2.1±0.6	7	4.8±0.8	t-value 3.39**
pollen grains per stigma	7	5.4±0.2	10	8.1±0.3	Mann-Whitney Z -7.04***
Scabiosa columbaria					
heads per minute	5	1.5±0.7	7	5.1±1.3	t-value -6.21***
pollen grains per stigma	13	3.7±0.1	4	6.1±0.4	Mann-Whitney Z -4.71***

*** = $p<0.001$; ** = $p<0.01$

Table 1: Foraging speed and pollen deposition (means ± SE) by syrphids (Eristalis tenax) and bumblebees (Bombus pascuorum), on Succisa pratensis and Scabiosa columbaria (n = number of insects).

Scabiosa columbaria

Visitors to *Scabiosa columbaria* were flies, bumblebees and butterflies, consuming pollen and/or nectar. Early in the season butterflies were more numerous than later on. The natural population was not visited by bumblebees, which were present in this area but concentrating on *Centaurea jacea*. During the day species composition of syrphids changed. Early, between 0900 and 1100 hours, *Episyrphus balteatus* was present, consuming pollen from the anthers; then this species disappeared but returned at about 1600 hours. *Eristalis*, *Helophilus* and *Volucella*

species were active between 1000 and 1700 hours.

The foraging speed of syrphids and bumblebees differed significantly: syrphids visited 1.5 flower heads per minute and bumblebees 5.1 (Table 1). The extent of dedication to foraging differed between syrphids and bumblebees as described for *S. pratensis*.

Pollen deposition by syrphids and bumblebees differed significantly. Syrphids (*Eristalis tenax* foraging for nectar) deposited 3.7 and bumblebees 6.1 pollen grains per stigma (Table 1). *E. tenax* individuals foraging for pollen only deposited 1.8 grains per stigma; these individuals searching for pollen left the female heads very quickly.

DISCUSSION

The floral biology and flower visitors of *P. nigrum* were alraedy studied by Sprengel in 1873. Most recently Kovanda (1981) supported his observations that bees, bumblebees, butterflies and flies were visitors. Westrich (1990) mentioned bumblebees, butterflies and several bee species as visitors of *P. nigrum*. Proctor and Yeo (1973) mentioned the syrphid *R. campestris* as visitor of *S. pratensis*. Bees as visitors of *S. pratensis* and *S. columbaria* were mentioned by Proctor and Yeo (1973) and Westrich (1990) and a sawfly (family Cimbicidae) was mentioned by Chambers (1947). For many, the flowers must be more important as food sources for the insects than the insects for pollination of the flowers.

Predominant visitors were not the most effective pollen vector. Total foraging time during the day, the number of flowers visited per minute, pollen deposition on stigmas, and seed set after visitation differed between syrphids and bumblebees; overall, bumblebees were better pollinators. Bumblebees, flies and butterflies are differentially sensitive to weather conditions (Heinrich 1979, Gilbert 1985). During relatively bad weather only bumblebees are still active. Especially when *P. nigrum* and *S. pratensis* are in flower (May-June and August until mid October), days with mainly or only bumblebee activity are frequent. That bumblebees can pollinate a whole plant population despite a low number of individuals was demonstrated in 1992 in population Geelbroek. The percentage of pollinated stigmas increased from 10 to 100 during the day when only bumblebees were visiting.

The pollination value of *R. campestris* to *P. nigrum* is very low. The densities of flies used in the cages was 0.5 and 2.0 flies per inflorescence. In the field we observed densities between 0.0003 and 0.0159 for 1991 and between 0.0022 and 0.0082 flies per inflorescence per count. Thus, densities used in the cages was much higher than in the natural situation. We did not find a negative effect of high syrphid density in the cages. Thus, bumblebees are the main pollinators of *P. nigrum*.

Flies had pollination value for *S. pratensis*, but the pollinating effectiviness of small flies was limited. Small species such as *Siphona geniculatus* hardly contributed to the pollination of *S. pratensis* (unpubl. data) in contrast to the syrphid *E. tenax* or *Helophilus pendulus*. Bumblebees deposited per stigma more grains than did syrphids, visiting either *S. pratensis* or *S. columbaria*. Taken into account not only the ratio of individual bumblebees and syrphids (1:15, counting only the larger sized syrphids as *Eristalis, Helophilus* and *Syrphus* s.l. visiting *S. pratensis* population Assen), but also foraging speed (ratio 1:0.4) and pollen deposition (ratio 1: 0.7), the

importance of bumblebees increases (ratio 1:4.4). For *S. columbaria* the same applies except that bumblebees were more numerous than on *S. pratensis* (ratio 1:7.6) in the experimental garden but absent in the natural population. In the experimental garden the pollination effectiveness ratio was 1:1.4.

Although both *S. pratensis* and *S. columbaria* set only one seed per flower and theoretically one grain per stigma should be sufficient, pollination by bumblebees is more profitable than that by syrphids. Several studies have shown that the number of pollen grains deposited on the stigma must exceed a minimum threshold for fruit set to occur (Waser and Fugate 1986, Herrera 1978).

In this study we measured pollination effectivity as seeds per flower in *P. nigrum*. The desired effect of a pollinator visit, from the plant's perspective, is seed production. Thus measuring seed set is a closer measure of plant fitness than is pollen deposition. Measuring pollen deposition by insects in *P. nigrum* is practically impossible due to the secondary pollen presentation with pollen adhering to the underside of the stigma. *P. nigrum* is strongly self-incompatible. On the other hand measuring seed set in *S. pratensis* and *S. columbaria* after insect visits is nearly impossible due to the very close arrangements of the flowers in the flower head, which makes individual marking of pollinated flowers very difficult. The possible difference in pollination quality between flies and bumblebees (differences in number and origin of pollen donors per individual insect) can be examined using allozyme variation in *S. columbaria* (see Van Treuren *et al.* 1993). Pollinator foraging behaviour may influence the pollen delivered to stigmas through its effects on average distance from the mother plant to the pollen source, and the number of pollen donors represented in the load.

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REFERENCES

DIERINGER, G., 1992 - Pollinator effectiveness and seed set in populations of *Agalinis strictifolia* (Scrophulariaceae). *Amer. J. Bot.* **79**,1018-1023.

CHAMBERS, V.H., 1947 - A list of sawflies (Hym., Symphyta) from Bedfordshire. *Entomologist's mon. Mag.* **83**,91-95.

GILBERT, F.G., 1985 - Diurnal activity patterns in hoverflies (Diptera, Syrphidae). *Ecol. Entomol.* **10**,385-392.

HEINRICH, B., 1979 - Bumblebee economics. Harvard University Press, Cambridge, Massachusetts, and London, England.

HERRERA, C.M., 1987 - Components of pollinator "quality": comparative analysis of a diverse insect assemblage. *Oikos* **50**,79-90.

INOUYE, D.W. 1980 - The terminology of floral larceny. *Ecology* **61**,1251-1253.

KOVANDA, M., 1981 - Studies in *Phyteuma*. *Preslia*, Praha **53**,211-238.

PROCTOR, M. & P.YEO, 1973 - The pollination of flowers. Collins, London.

SPEARS, E.E., 1983 - A direct measure of pollinator effectiveness. *Oecologia* **57**,196-199.

SUGDEN, E.A., 1983 - Anthecology and pollinator efficacy of *Styrax officinalis* subsp. *redivivum* (Styracaceae). Amer. J. Bot **73**,919-930.

VAN TREUREN, R., R. BIJLSMA, N.J. OUBORG & W. VAN DELDEN, (1993) - The effects of population size and plant density on outcrossing rates in locally endangered *Salvia pratensis*: *Evolution*, in press.

WASER, N. & M.L. FUGATE, 1986 - Pollen precedence and stigma closure: a mechanism of competition between *Delphinium nelsonii* and *Ipomopsis aggragata*. *Oecologia* **70**,573-577.

WASER, N.M. & M.V. PRICE, 1990 - Pollination efficiency and effectiveness of bumblebees and hummingbirds visiting *Delphinium nelsonii*. *Collect. Bot.* (Barcelona) **19**,9-20.

WESTRICH,P., 1990 - Die Wildbienen Baden-Württembergs, Spezieller Teil: Die Gattungen und Arten. Eugen Ulmer GmbH & Co, Stuttgart.

OVERWINTERING OF *LIRIOMYZA BRYONIAE* AND *LIRIOMYZA HUIDOBRENSIS* (Diptera: Agromyzidae) IN THE NETHERLANDS

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Key words: Liriomyza bryoniae, Liriomyza huidobrensis, overwintering

Summary

The ability of *Liriomyza bryoniae* (Kaltenbach) and *Liriomyza huidobrensis* (Blanchard) pupae to overwinter outdoors was investigated in the winters of 1990/1991 and 1991/1992. Although mortality of overwintering pupae was high in comparison with directly reared pupae, part of both *L. bryoniae* and *L. huidobrensis* survived a winter with 30 frost days and the lowest minimum of - 11.5°C.

INTRODUCTION

Liriomyza bryoniae (Kaltenbach), the tomato leaf miner, is a pest in glasshouses. This leafminer occurs in Europe, islands included (Spencer, 1973, 1990); Russia (Safjanov & Skripnik, 1968), Japan (Kamijo, 1978), Taiwan (Wang & Lin, 1988); Egypt and Morocco (Spencer, 1973). Spencer (1990) revised the list of hosts, which comprises hosts in 16 families.

Liriomyza huidobrensis (Blanchard) is native in much of temperate South America: Colombia, Venezuela, Peru, Brazil, Argentina, Chile and Costa Rica; North America: U.S.A. (California, Utah and Washington) (Spencer, 1973, 1982; Spencer & Steyskal, 1986). Hosts in 14 families are listed (Spencer, 1990).

Both leafminer species are very closely related. In the laboratory crossing of the two species was possible. The offspring showed an intermediate hybrid (Van der Linden, unpublished).

In South America chemical control of *L. huidobrensis* has become difficult because of resistance. For example, in Peru *L. huidobrensis* developed into a primary pest in potato as a result of indiscriminate spraying against another pest, the moth *Scrobipalpula absoluta* (Lepidoptera: Gelechiidae) (Raman, 1988).

L. huidobrensis is a pest in The Netherlands and other European countries since 1989, and appeared harmful in many crops in glasshouses as well as outside (Van der Linden, 1990 a). In view of the fact that chemical control of this species in Europe is difficult, its origin is likely to be somewhere in South America rather than in North America, since there are no problems with chemical control reported there.

Since it was questioned wether *L. huidobrensis* could survive during winters in The Netherlands observations were carried out on both *L. bryoniae* and *L. huidobrensis* in the winters of 1990/1991 and 1991/1992.

MATERIALS AND METHODS

Tomato plants were infested with *L. bryoniae* and *L. huidobrensis* in maintenance rearings in glasshouse compartments during seven days. Pupae were collected ten to fourteen days after the infestation period. Temperature was set at 18°C and ventilation at 23°C. Additional light was received from adjacent glasshouse compartments (dark/light period 8/16 h). Collection dates and numbers of pupae are given in Table 1. Half the number of pupae per batch was directly reared to the adult stage, the other half was put in glass tubes and these were put in a glass jar (17 cm diameter, 30 cm high). The jar was placed outside at the northside of a building to prevent rising temperatures solar radiation. The opening was sheltered from rain and snow by a lifted cover allowing continuous ventilation.

Table 1. Collection dates and number of pupae of Liriomyza bryoniae and Liriomyza huidobrensis. Half the number per batch was directly reared, the other half overwintered.

Collection date	L. bryoniae	L. huidobrensis
29-11-1990	200	50
3-12-1990	220	200
18-12-1990	200	200
21-12-1990	280 +	140 +
total 1990	900	590
26-11-1991	200	220
28-11-1991	260	50
16-12-1991	540	300
19-12-1991	460	500
30-12-1991	320	660
2- 1-1992	400 +	400 +
total 1991	2180	2130

Of both the directly reared and overwintered pupae the number of emerging adults was counted. Mortality percentages were calculated as follows:

$$\% \text{ mortality} = \frac{\text{non-emerged pupae}}{\text{total pupae}} \times 100\%.$$

The adjacent weather station provided computerized recording of weather conditions (Table 2). Temperatures ware also checked roughly once per week by means of a maximum/minimum thermometer in the glass jar.

RESULTS AND DISCUSSION

Table 3 shows that part of the overwintered pupae of both *L. bryoniae* and *L. huidobrensis* survived, although mortality was much higher in comparison with directly reared pupae. Nowakowski (1962) stated that dark-coloured pupae of several leafminer species represent the

hibernating generation. Helyer & Ledieu (1990) found that adults of *L. bryoniae* emerged earlier from light-coloured pupae than from dark-coloured ones, whilst emergence of adults from the medium coloured pupae was intermediate. Dark-coloured pupae with a naturally longer pupal stage might indeed be better able to hibernate.
The overwintering pupae in this study were not prepared for overwintering. Although light intensity was not high in the leafminer rearings, long daylength was probably responsible for the formation

Table 2. Number of frost days; monthly minimum, maximum and mean temperatures; and percentage relative humidity (%RH) in the winters of 1990/1991 and 1991/1992.

	Number of frost days	Min. temp. °C	Max. temp. °C	Mean temp. °C	%RH
Dec 1990	4	- 1.6	10.7	5.4	81
Jan 1991	8	- 2.5	12.9	4.3	83
Feb 1991	18	- 11.5	12.8	0.8	89
Mar 1991	0	2.7	18.5	9.2	79
Apr 1991	0	1.1	23.1	9.2	73
May 1991	0	5.2	18.4	10.0	79
Dec 1991	3	- 2.8	12.0	5.3	88
Jan 1992	9	- 5.7	10.8	3.8	92
Feb 1992	3	- 1.6	15.2	6.1	89
Mar 1992	0	1.1	15.0	7.4	87
Apr 1992	0	1.2	16.4	9.4	80
May 1992	0	5.4	30.5	15.6	71

of light coloured pupae. Those of *L. bryoniae* were light brown with few exceptions, and those of *L. huidobrensis* were slightly darkened but not black. Apparently light and medium coloured pupae were not necessarily unable to survive during winter.

The observation that *L. huidobrensis* was able to survive frost, is supported by Leuprecht (1991), who reared adults from pupae collected outside in Germany after a period of frost with the lowest recorded temperature of - 9.1°C.

It was striking that many overwintering pupae contained developed adults, which apparently died shortly before emergence. Keularts & Lindquist (1989) reported that low relative humidity caused increased mortality of *Liriomyza trifolii* pupae. However, Table 2 shows that the mean relative humidity was not low during the observations. In a natural situation the pupae lay upon the soil or just below the soil surface. It is possible that leafminer pupae actively absorb water to prevent drying up. During these observations the pupae were kept seperate from water.

Prando & Da Cruz (1986) found an average mortality of 15.4%in directly reared *L. huidobrensis* pupae from beans (*Phaseolus vulgaris*) (60-80% RH). Parrella & Bethke (1984) found 64.0% mortality in *L. huidobrensis* pupae from chrysanthemum, 71.0% from *Aster* and 26.2 from peas (50-60% RH). Table 3 shows that tomato belongs to the moderate to good host plants with 34.2% and 22.5% mortality. Adaptation to a particular host plant after many generations might improve survival on

that host.

Overwintering pupae of *L. huidobrensis* emerged earlier than those of *L. bryoniae*. Table 4 shows the dates on which 50% of the emerged adults were counted. In general 50% of the *L. bryoniae* adults had emerged at the end of May and *L. huidobrensis* in the first half of March independent of the date of collection. Pupae of *L. huidobrensis* collected on 16th and 19th December 1991 tended to emerge earlier than the other pupae.

Table 3. Percentage mortality of directly reared and overwintered pupae of Liriomyza bryoniae and Liriomyza huidobrensis in the winters of 1990/1991 and 1991/1992.

Collection date	% Mortality *L. bryoniae*		% Mortality *L. huidobrensis*	
	direct	wintered	direct	wintered
29-11-1990	1.0	64.0	68.0	100.0
3-12-1990	0	72.7	35.0	97.0
18-12-1990	35.0	94.0	22.0	90.0
21-12-1990	56.4	91.4	38.6	100.0
Mean mortality	25.6	81.3	34.2	95.6
26-11-1991	39.0	66.0	0	96.4
28-11-1991	16.2	59.2	24.0	88.0
16-12-1991	30.0	79.3	30.7	92.7
19-12-1991	19.6	77.4	24.8	84.8
30-12-1991	32.5	85.0	23.3	82.4
2- 1-1992	42.0	91.0	24.5	83.5
Mean mortality	29.5	78.3	22.5	86.2

Table 4. Collection dates of pupae, the 50% emergence dates of Liriomyza bryoniae and Liriomyza huidobrensis and the duration of the pupal stage in days.

Collection date	*L. bryoniae*		*L. huidobrensis*	
	50% emergence date (days)	pupal stage (days)	50% emergence date (days)	pupal stage (days)
29-11-1990	31-5-1991(183)	146-207	-	-
3-12-1990	31-5-1991(179)	142-193	15-3-1991(102)	102-112
18-12-1990	31-5-1991(164)	135-178	15-3-1991(87)	83- 93
21-12-1991	13-6-1991(174)	161-202	-	-
26-11-1991	27-5-1992(183)	174-196	9-3-1992(104)	104-111
28-11-1991	25-5-1992(179)	172-188	11-3-1992(104)	81-104
16-12-1991	27-5-1992(163)	28-168	17-2-1992(63)	7- 84
19-12-1991	25-5-1992(158)	81-165	17-2-1992(60)	18- 81
30-12-1991	27-5-1992(149)	49-156	9-3-1992(70)	49- 91
2- 1-1992	27-5-1992(146)	60-153	11-3-1992(69)	46- 88

In natural outdoor conditions hibernation of pupae will start at the end of the summer and during autumn, and will last until the next year. Even in the glasshouse (15°-25°C, natural daylength) the life cycle of *L. bryoniae* begins to extend rapidly during August, and after November this phenomenon gradually disappears again (Helyer & Ledieu, 1990). They concluded that this would indicate photoperiod rather than temperature as the critical factor responsible for the period of extended pupation. Similar conclusions were drawn for the extended pupation period of a natural enemy of *Liriomyza* spp., the endoparasitoid *Dacnusa sibirica* (Hymenoptera: Braconidae) (Van der Linden, 1992). Although photoperiod is probably the critical factor, temperature is not completely irrelevant. Storage of *L. bryoniae* pupae in the refrigerator at 7°C was sufficient to prevent emergence of adults. A small part of the pupae still survived storage for 160 to 300 days, and adults emerged when the pupae were brought to 23-27°C (Van der Linden, 1990b). Short daylength and low temperatures will probably show synergism on the period of extended pupation. Each species may have a different temperature threshold above which it needs a certain temperature sum to complete its development.

In natural situations mortality of overwintering pupae might be lower than in these observations, because pupae are probably better prepared for hibernation. There is possibly also less chance of drying up, while on the other hand wet conditions might increase mortality by fungi. Anyhow, the conclusion of this study is that both *L. bryoniae* and *L. huidobrensis* are able to overwinter outdoors in The Netherlands and may infest crops again in the next year.

REFERENCES

HELYER, N & M. LEDIEU, 1990 - The seasonal variability in tomato leafminer (*Liriomyza bryoniae* (Kaltenbach)) life cycle. *SROP/WPRS Bull.* **XIII/5**: 83-86.

KEULARTS, J.L.W. & R.K. LINDQUIST, 1989 - Increase in mortality of prepupae and pupae of *Liriomyza trifolii* (Diptera: Agromyzidae) by manipulation of relative humidity and substrate. *Environ. Entomol.* **18** (3) : 499-503.

KAMIJO, K., 1978 - Chalcidoid parasites (Hymenoptera) of Agromyzidae in Japan, with description of a new species. *Kontyû*, Tokyo, **46** (3) :455-469.

LEUPRECHT, B., 1991 - Die Minierfliege *Liriomyza huidobrensis*. *Gemüse* **27** (7): 362-365.

LINDEN, A. VAN DER, 1990[a] - Prospects for the biological control of *Liriomyza huidobrensis* (Blanchard), a new leafminer for Europe. *SROP/WPRS Bull* **XIII/5** : 100-103.

LINDEN, A. VAN DER, 1990[b] - Survival of the leafminer parasitoids *Chrysocharis oscinidis* Ashmead and *Opius pallipes* Wesmael after cold storage of host pupae. *Med.Fac.Landbouww.Rijksuniv .Gent*, **55** (2a): 355-360.

LINDEN, A. VAN DER, 1992 - *Phytomyza caulinaris* Hering, an alternative host for the development of an open rearing system for parasitoids of *Liriomyza* species. *Proc.Exper.& Appl.Entomol., N.E.V. Amsterdam,* vol. 3 : 31-39.

NOWAKOWSKI, J.T., 1962 - Introduction to a systematic revision of the family Agromyzidae (Diptera) with some remarks on host plant selection by these flies. *Ann.zool., Warzawa* **20** (8) : 67-183.

PARRELLA, M.P. & J.A. BETHKE, 1984 - Biological studies of *Liriomyza huidobrensis* (Diptera: Agromyzidae) on Chrysanthemum, Aster, and pea. *J.Econ.Entomol.* **77** (2) : 342-345.

PRANDO, H.F. & F.Z. DA CRUZ, 1986 - Aspectos da biologia de *Liriomyza huidobrensis* (Blanchard, 1926) (Diptera: Agromyzidae) em laboratório. *An. Soc. Entomol. Brasil*, **15** (1): 77-88.

RAMAN, K.V., 1988 - Integrated insect management for potatoes in developing countries. *CIP Circular* **16** (1) : 1-8.

SAFJANOV, S.P. & J.J. SKRIPNIK, 1968 - [The solanaceous miner]. *Zashchita Rastenii* **13** (12) : 51. (Russian)

SPENCER, K.A., 1973 - Agromyzidae (Diptera) of Economic Importance. *Series Entomologica*, vol. **9**. Junk, The Hague. 418 pp.

SPENCER, K.A., 1982 - Review of Colombian leafminers with special reference to *Liriomyza huidobrensis*. In Poe, S.L. *Proceedings of the 3rd Annual Industry Conference on the leafminer*. Nov. 8-9-10, 1982. Sheraton Inn - Airport, 1590 Harbor Island Drive, San Diego, CA, p. 101-103.

SPENCER, K.A., 1990 - Host specialization in the world Agromyzidae (Diptera). *Series Entomologica* **45** : 1-444. Kluwer Academic Publisher, Dordrecht, Boston, London.

SPENCER, K.A.& G.C. STEYSKAL, 1986 - Manual of the Agromyzidae (Diptera) of the United States, *U.S. Department of Agriculture, Agriculture Handbook* no. **638**, 478 pp.

WANG, C.L.& F.C. LIN, 1988 - A newly invaded insect pest *Liriomyza trifolii* (Diptera : Agromyzidae) in Taiwan. *Journal of Agricultural Research of China* **37** (4) : 453-457.

SIMULATING DWELLING CONDITIONS FOR MITE-POPULATION STUDIES, A LABORATORY MODEL

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Key words: house dust mites, storage mites, population studies, indoor environment, *Dermatophagoides pteronyssinus*, *Glycyphagus domesticus*

Summary

A semi-natural laboratory model was developed, in which the development of mites in dwellings may be studied. Optimum and minimum conditions of 4 different organisms, representative for 2 groups of mites living in dwellings, were determined on 4 different subsoil materials with 2 types of artificial soiling, thus representing floors, textile surfaces and walls. House dust mites develop readily on wood, gypsum, carpet, or mattress models, soiled with a mineral/organic mixture (1:1) at 75% relative humidity and room temperature, but not at 60% humidity or on a light 100% organic soiling. On that material storage mites prosper in the presence of fungal growth, and when humidity is sufficiently high. Use of acaricidal products resulted for two acaricides in a decreased population; Of a third product no reducing effect could be determined. However, prolonged incubation resulted in a thriving population in 4 out of 8 tests. Studies in dwellings corroborate these results. As it is possible to set parameters like temperature, relative humidity, subsoil, soiling, type and number of species and coappearing organisms, this model is useful for the stationary simulation of many different dwelling conditions for mite population studies.

Introduction

In dwellings, house dust mites and storage mites inhabit the soiled surfaces of floors and furniture (Bronswijk 1981). Recently has been discovered that both groups also live on different types of walls (Kort 1990). These organisms affect the health of inhabitants by producing allergenic substances, and some disfigure textiles and wall material. They can only prosper in favourable conditions, which means a high relative humidity and usually a temperature between 20 and 30 °C. The climate in dwellings often satisfies these requirements. Each species has a specific range of temperature and humidity, in which reproduction can take place, and each has its own requirements for the substrate, for both nutrition and niche.

Reduction of domestic mites is pursued by dissatisfying to vital conditions, including lowering indoor humidity, removal of niches, and by mechanical or chemical intervention. The mechanisms and efficacy of each procedure may be studied in dwellings,which are less reproducible, or in direct-effect tests. These tests, however,

lack in validity, because dwelling conditions are disregarded.

A semi-natural environment was developed that resembles in many ways the situation in non-industrial buildings. Thus the population growth of mites may be followed, and, unlike the circumstances in buildings, the installed components and conditions are in all experiments identical, thus rendering more reproducible results. Furthermore, the effects of varying one parameter may be analyzed in this controlled environment.

Material and methods

The heart of the semi-natural environment is a piece of material of 10 by 10 cm, 6 to 10 mm thick. The material of each piece is representative for a furnishing or finishing material used in dwellings. We have chosen four different materials: unfinished deal-wood, and gypsum building board without protective paper, representing wall material; polyamide carpet with a polyester foam cover; and a hand-made mattress model, compiled from two layers of polyurethane foam, 4 mm thick, between two layers of an acetate/viscose mattress tick, which are stitched with zigzag stitches along the edges and straight stitches across the surface.

The soil layer that is found on horizontal surfaces in dwellings is mimicked using 0.5 to 1.0 g artificial dust that is spread over and pressed into the surface of the test piece. The dust consists of 50% mineral powders, as used in carpet industries for cleaning tests, and 50% organic material, i.e. 1 part milled yeast and 3 parts dander. Thus, the artificial dust has a texture and chemical composition as might be found in floor or mattress dust (Schober 1991). This composition is much less representative for wall surfaces. These surfaces have a lower storage capacity and only light, airborne dust is found here. For wall surfaces, a largely organic artificial soiling is more suitable. In an experiment with a yeast/vegetable extract mix with a defined composition (Marmite), this substrate, 0.2 g per piece, was tested for its qualities as a medium for culturing house dust mites and storage mites. In dwellings these latter mites mainly feed on fungi; Therefore the development of fungi on this type of soiling has been examined too (Table 1).

The test pieces are laid in Petri dishes, which are subsequently covered with a poplin (batiste) fabric. Quickfooted organisms are kept inside by applying a sticky substance (Tangle Trap, Tanglefoot Cy., Michigan) to the upper edges of the dish. The test environment is restricted to airtight polythene bags (Polypax, Baarn, the Netherlands, air exchange 1 g/m^3/24hrs). Relative humidity inside is constant, due to a beaker with a super-saturated salt solution and a moisture buffering cardboard. The bags are placed in a thermostated room. Temperature and humidity are controlled by a hair hygrometer and a hygroscope (Rotronic AG, Zurich, Switzerland) that is placed in one of the bags. Eight dishes may be stacked in each bag. Standard values for humidity and temperature are respectively 75% ($\pm$ 2) R.H. and 20 ($\pm$ 1) °C.

Before incubating organisms, the system is acclimated for some weeks. A pilot study showed that the relative humidity of the materials reaches equilibrium after two to four weeks. The incubation period of the organisms varies depending on the rate of development of the species at the established temperature and humidity.

Of house dust mites, species *Dermatophagoides pteronyssinus* or *D.farinae*, 50 individuals are randomly picked one by one from a developing culture. The mites are

usually raised on a nutrient medium equal to the soiling substrate. This reduces the number of mites that is unable to comply with changing conditions. Under favourable conditions, fungi-feeding storage mites of the species *Glycyphagus domesticus* or *Tyrophagus putrescentiae* are developing more quickly than house dust mites. Under those circumstances only 20 organisms of these species are incubated to prevent overcrowding at the end of the incubation. In only a few days the mites have entered and accepted their niche.

The population development is continued for 8 weeks after application of a certain measure. Under normal conditions this period is approximately two life cycles of both storage mites and house dust mites. Dishes are ventilated every two weeks to avoid carbon dioxide accumulation. All tests are run in duplicate and in two, in time and space separated series. Blanks are added for the determination of undisturbed population development.

The development of the mites is determined by counting, usually after a heat-escape method (Bischoff *et al.* 1986). In this procedure each test piece is covered with a transparent adhesive plastic sheet, and is placed on a 60 °C plate. The living mites fly from the heat to the top and stick to the adhesive cover. The number of living mites is counted on the sheet. The dust layer may be vacuumed off for assessment of the quantity of allergens or of guanine, which gives information on the total number of mites that lived on the test piece.

The rate of development of inoculated or contaminating fungi is determined by a visual quantification of the two-dimensional growth of hyphae. A microscope at 10x magnification is used. Of each square cm of the test piece the area of fungal growth is graded as: 0 (no visible fungal growth), 1 (light fungal growth, 1-50% of the surface covered with hyphae) or 2 (heavy fungal growth, 51-100% surface covered).

The significance of results may be determined with a non-parametric two-sample test, e.g. the Mann-Whitney U test. Limit of confidence is set at $p = 0.05$.

Results

Starting with 50 mites, 8 weeks after incubation 100 to 700 house dust mites may be found on undisturbed standard test pieces that are soiled with artificial floor/mattress dust. The type of material is usually not important, though wooden pieces with resinous spots may enhance growth. Acclimatization shorter than 14 days resulted on some pieces, especially gypsum, in extinction of the population. Populations of *D.pteronyssinus* mites in a 55-60% R.H. environment did not survive 8 weeks of incubation. Storage mites *Glycyphagus domesticus* are developing at 75 to 80% R.H. at similar speed as *D.pteronyssinus*, 100 to 400 mites could be found.

On wood and gypsum board pieces soiled with organic material, thus representing wall surfaces, at first *Aspergillus repens* was inoculated (1 million spores in 0.1 ml water) and after 6 weeks 50 mites, either *D.pteronyssinus* or *G.domesticus*, were added. 8 weeks later mites and fungi were counted. On the soiled not-inoculated pieces, contaminating fungi (*Aspergillus* and *Penicillium* species) were growing, only a little bit less than the inoculated *A.repens*. House dust mites seem not to favour this material, whereas storage mites are prospering (*Table 1*).

Table 1. Development of fungi Aspergillus repens (Saccardo), storage mites Glycyphagus domesticus (De Geer), and house dust mites Dermatophagoides pteronyssinus (Trouessart) on wood and gypsum board soiled with a yeast/vegetable extract mix. Fungal growth area (max. = 200) and mite numbers (average resp. median of 4) after a 14- respectively 8-week incubation at 20 °C and 80% relative humidity.

material:	WOOD		GYPSUM	
organism	fungal growth	number of mites	fungal growth	number of mites
A.repens only	27	--	2	--
soiling only	112	--	176	--
soiling + *A.repens*	155	--	197	--
soiling, *A.repens* + *D.pteronyssinus*	157	9	157	5
soiling, *A.repens* + *G.domesticus*	107	290	84	391

The semi-natural system has been used to predict under home circumstances the acaricidal efficacy of some products that were sold as acaricides. Model mattresses were soiled with 1 g artificial mattress/floor dust, and development of *D.pteronyssinus* was followed after treatment with either Tymasil (3.33% natamycin) or Allersearch (benyltannate alcohol complex) or Acarosan (wet powder formula, containing 5% benzyl benzoate). Eight weeks after treatment, development varied per product (*Figure 1*). Tymasil had no effect on *D.pteronyssinus* compared to undisturbed development, whereas both Allersearch and Acarosan did decimate the populations significantly ($p = 0.03$ resp. 0.014). In a prolonged experiment, mite development was followed for 24 weeks. Blank development varied from 1400 to 6000 mites, whereas the treated populations showed a much wider range. Only treatment with the Acarosan formula resulted in a significantly lower development than the blank ($p=0.014$).

Discussion

In studies in dwellings house dust mite populations increased at humidities over 12 g/m3, which is equivalent to approximately 70% RH at 20 °C, and diminished under this value (Lustgraaf 1975, Dusbabek 1975). In semi-natural experiments at 20 °C in our laboratory, house dust mites appeared to survive and even thrive on soiled materials when the relative humidity was 75% or higher, i.e. a water vapour concentration of 13 g/m^3. A population of mites in a 55-60% R.H. (10 g/m^3) environment did not survive

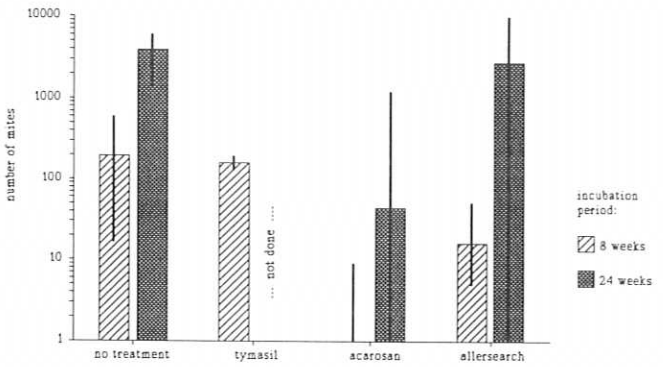

Figure 1. Median number of living mites D.pteronyssinus , 8 resp. 24 weeks after acaricidal treatment. Temperature was 20 °C, relative humidity was 80%, starting number of mites was 50. Vertical bars indicate range.

8 weeks of incubation.

In wall samples, the coappearance of storage mites and xerophilic or mesophilic fungi was determined (Kort 1990). House dust mites were found in only a few samples. The results from present experiments indicate that set conditions are less favourable for these mites. The lesser shielding capacities of this niche, as well as the presence of fungi may contribute to the reduced vitality of the population.

Irregularly, contaminating fungal growth appears when relative humidity is set at values above 75%. The fungi affect the vitality of the mite populations, and thus the outcome of the tests. Prevention of this phenomenon by sterilization of the subsoil and dust has up to now been impossible, as all sterilization techniques (heat, alcohol, microwaves) affect the nutritive and sheltering capacity of the materials.

The semi-natural model may at present only be used for stationary situations. Parameters like temperature and relative humidity may be set at will. For instance, on basement floors lower temperatures and higher humidities than the values used here, might be more appropriate. However, fluctuating temperatures and humidities, as in day/night rhythms, have not yet been tried with supersaturated salt solutions and may be less practicable.

From experiments with acaricides in dwellings, controversial results were obtained. Acarosan powder e.g., was determined to be both an efficient mite killing agent (Morrow Brown, Merrett 1991), and a non-effective one (Huss *et al.* 1992). In present paper both decimated and relatively thriving populations were found after treatment in identical situations, just depending on the time of observation.This indicates that the product may have a more limited effective time-span under certain circumstances than the period claimed by the manufacturer (6 months).

The results from these experiments, corroborated by results obtained from dwellings, confirm that present model is a useful tool for mite studies in dwellings.

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References

BISCHOFF, E., & A. FISCHER & G. WETTER, 1986 - Untersuchungen zur Oekologie der Hausstaubmilben. *Allergologie* **9**(2),45-54

BRONSWIJK, J.E.M.H. V., 1981 - House dust biology for allergists, acarologists and mycologists. NIB publ., Zeist NL, pp.34-44

DUSBABEK, F., 1975 - Population structure and dynamics of the house dust mite, *Dermatophagoides farinae* (Acarinae: Pyroglyphidae) in Czechoslovakia. *Folia Parasitol.* **22**(3),219-231

HUSS, R.W., *et al.*, 1992 - Mite control with acaracide fails. Abstract, *J.All.& Clin.Immunol.* **89**(1),316

MORROW BROWN, H, & T.G. MERRETT, 1991 - Effectiveness of an Acaricide in management of house dust mite allergy. *Annals of Allergy* **67**,25-31

KORT, H.S.M., 1990 - Mites, dust lice, fungi and their interrelations on damp walls and room partitions. *Proc. Exper.& Appl. Entomol.* **1**,63-68

LUSTGRAAF, B.V.D., 1978 - Seasonal abundance of xerophilic fungi and house dust mites (Acaridae; Pyroglyphidae) in mattress dust. *Oecologia* **36**,81-91.

SCHOBER, G., 1991 - Synthetischer Hausstaub als Substrat für allergenerzeugende Hausstaubmilben *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). *Allergologie* **14**,140-143

OVIPOSITION PREFERENCE IN RELATION TO FOOD PLANT SUITABILITY IN THE BUTTERFLY *BICYCLUS ANYNANA* (Satyrinae)

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Key words: oviposition, larval performance, egg size, seasonal polyphenism

Summary

The oligophagous tropical butterfly *Bicyclus anynana* accepts a wide range of grass species in oviposition and larval performance experiments: the more preferred species appear to be the most suitable food plants as deduced from survival rate, development time and pupal weight. A non-grass monocotyledon species *Cyperus cyperoides* is accepted at a very low rate. Food plant quality may influence egg size, as female weight is positively correlated with the size of the eggs she lays. However, egg size is not related to egg hatching. The effect of food plant quality on the seasonal polyphenism of *Bicyclus anynana* is discussed.

INTRODUCTION

The evolution of phenotypic plasticity in the form of seasonal polyphenism is being studied in African butterflies of the genus *Bicyclus* (Satyrinae) (Brakefield & Reitsma, 1991, Windig, in press.). The adult butterflies are present throughout the year. A life cycle scenario for polyphenic tropical Satyrine butterflies in highly seasonal environments is outlined by Brakefield (1987). In the dry season courtship behaviour and oviposition may be absent or only occur at a low level; in the wet season the reproductive success is optimized by an active adult life with a relatively rapid mating and subsequent oviposition on the adequate or abundant supply of grass food plants. Acceptance by the insect of various food plants combined with an ability of females to select the more suitable ones for oviposition, could promote shifts to available, suitable host plants throughout the wet season.

The present investigation of the oligophagous grass foliage chewing species *B. anynana* examines whether this species is able to select the more suitable food plants; oviposition choice and food acceptance are compared under laboratory conditions.

In many studies on host plant selection, the fresh weight of pupae is taken as a non-destructive measure to express the quality of larval food as positive relationships occur between pupal weight and fecundity. As a first approach to analyzing this relationship in *B. anynana* information on the correlation between female pupal weight and egg size is collected.

MATERIAL AND METHODS

B. anynana was collected in Malawi (see Brakefield & Reitsma, 1991) and maintained for more than twenty generations on *Zea mays* L.

In oviposition experiments females were tested with the following potential host plants (tropical grasses) with freshly green leaves: *Oplismenus undulatifolius* (Ard.) Beauv. = (Ou), *Oplismenus compositus* (L.) Beauv. var. *rariflorus* (Presl.) U. Scholz (=Oc), *Ganotia stricta* Brongn. var. *longiseta* Hackl.(=Gs), *Setaria palmifolia* (J.G. Koenig) Stapf (=Spa), *Setaria* spec. (=Ss), *Setaria plicata* (Lamk.) Cooke (=Sp), *Axonopus flexuosus* (Peter) Troupin (=Af), *Zea mays* (=Zm), *Digitaria setifera* R. et S. (=Ds) and *Panicum monticola* Stapf (=Pm). The plants were grown in a greenhouse (L16 : D8). As the plants differed in size the dry weight of their leaves was measured.

The oviposition tests were performed in two climate controlled rooms (23°C, L12:D12) (5.9 x 2.25 x 1.9 m = room A, 1.5 x 0.6 x 0.6m = room B). For the number of plants, trials and butterflies used in the tests see fig. 1. After four to seven days the number of eggs on each plant species was counted.

In larval performance experiments newly emerged first instar larvae were fed grasses at 28°C and 20°C, L12:D12, rh. 90-100%. At 28°C twelve groups of five larvae were placed in small net cages (3,5 cm height, 12 cm diam.) fastened on Oc, Sp or Ds. At 20°C eight groups of thirty larvae were placed on Oc and sixteen groups of thirty larvae on Pm in a gauze sleeve.

Supplementary observations were made on the acceptance of the following non-grasses: *Cyperus cyperoides* (L.) Kuntze subsp. *flavus* K. Lye (=Cc), *Juncus* cf. *effusus* L. (=Je), *Tradescantia repens* Vand. (=Tr) (its leaves look like those of Oc), *Pisum sativum* L. (=Ps) and *Vicia faba* L. (=Vf).

Observations were made on the diameter of the first laid eggs by females (their pupal weight was known) at 28°C with a stem of Oc (maximal fifteen eggs per female). The egg-hatching was observed on $\pm$ 400 hundred eggs from a stock of *B. anynana* and for which the diameters were measured within 24 hours after laying. Egg diameter is a good measure for egg weight (Van Oosterhout *et al.*, this volume).

RESULTS

Eggs were laid on all of the grass species in the oviposition tests (fig. 1). A significant difference in the number of eggs in the test with ten species is found (ANOVA, $F=9.6$, $df = 9$, $P < 0.001$); no differences occurred between the various trials (P is N.S. in each case). When the number of eggs on the ten plants in this test are compared with the other tests in fig. 1, a more or less consistent pattern is found; the more preferred species of the ten plants are also the preferred ones in the other tests, both in rooms A and B. The number of eggs on each plant within a trial was not correlated with its dry weight.

The larvae developed well on Oc, Sp and Ds at 28°C. Females and males differed significantly in developmental period (ANOVA, $F = 29.76$, $df = 1$, $P<0.001$) and pupal weight ($F = 98.78$, $df = 1$, $P<0.001$). Oc is the most suitable food plant as the development time was shorter ($F = 74.17$, $df = 2$, $P< 0.001$) and the pupal weight significantly higher ($F = 14,56$, $df = 2$, <0.001); no difference in survival rate occurred (Chi-square test, $\chi^2 = 1.85$, $df = 2$, $P>0.05$). Large differences in these

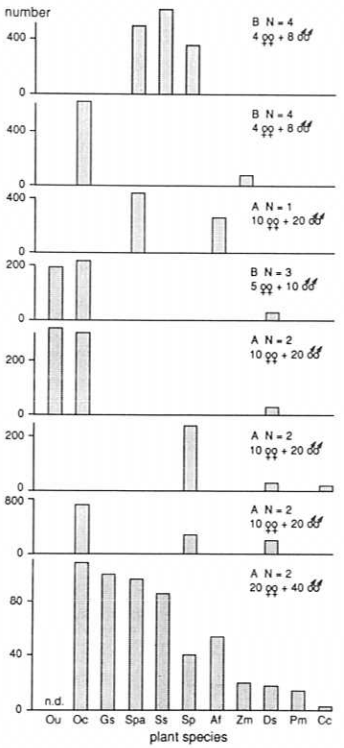

Fig. 1. Number of eggs deposited in different tests on various plant species in room A or B. Each histogram gives the pooled results of N trials of a test. The number of butterflies used per trial is noted for each test. For abbreviations of plant names see text. In the final test ten of the species were used; in the other tests two or three.

parameters were found for Oc and Pm at 20°C, indicating that Pm is scarcely suitable as a food plant.

In the oviposition tests a low number of eggs were laid on the non-grass Cc (fig. 1), while when this plant was offered in a no choice situation many eggs were laid on it. A comparable observation occurred with Je; few egg were laid in a choice situation and many more when it was offered without grass species. Hardly any eggs were laid either in a choice, or a no-choice situation on Tr, Ps and Vf. When non-grasses were offered as food plant to (an unknown large number) either first or fourth instar larvae

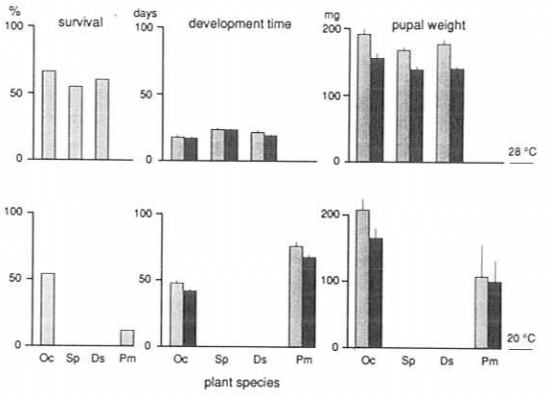

Fig. 2. Larval performance on Oc, Sp and Ds at 28°C and on Oc and Pm at 20°C (means ± SE). Grey = females, black = males. For abbreviations of plant names see text.

the larvae were only able to complete their development on Cc, albeit with a very low survival rate (< 5%), a long developmental period and a low pupal weight (< 110 mg).

An ANOVA shows significant differences in egg size between individual females $F = 8.57$, $df = 26$, $P < 0.001$). The eggs of large females are significantly larger than those of smaller females. This positive relationship between pupal weight and egg size is found for the relationship when the mean egg diameter per group is analysed (Pearson corr. coëff., $n = 27$, $r = 0.367$, $P < 0.05$) and also with the individual values of every egg (n of eggs = 366, $r = 0.163$, $P < 0.01$). The mean diameters of hatched and non-hatched eggs are identical (= 0.49 mm).

DISCUSSION

Some grass-feeding Lepidoptera including some species of Satyrines lay their eggs in sites near but not on the host plants (Bernays & Barbehenn, 1987, Wiklund, 1984). Most Satyrines do lay directly on grasses. *B. anynana* will lay eggs away from grasses on leaves in the vicinity of grass foliage in free-flying tropical greenhouse populations. Butterflies have been observed laying eggs on grasses including Oc, Af and, on one occasion, on Cc in the field in Malawi (P.M. Brakefield, pers. comm.). As eggs were laid in the present study on the unsuitable food plant Je the plant recognition by *B. anynana* is apparently not perfect under laboratory conditions. The insects were raised on Zm for more than twenty generations prior to the oviposition experiments but a preference for Zm was not developed.

A more or less consistent pattern is found in the oviposition experiments in the different environments (room A and B). The results are in agreement with the larval performance experiments: the most preferred plant species appears to be the most suitable food plant indicating that *B. anynana* is able to select suitable food plants. The suitability of Oc as a food plant corresponds with earlier observations performed at

17°C and 28°C (dry and wet seasonal conditions, respectively) (Kooi, in press). The acceptance of Cc corresponds with the observation that species of sedges are sometimes included in the diet of graminivorous. Observations and tests of feeding on other closely related monocotyledones have rarely been made (Bernays & Barbehenn, 1987).

The insect laid no eggs and could not survive on the dicotylodones offered. The monocotyledones can be divided into: A) Grasses which differ both in their acceptability in the oviposition experiments and also in their suitability as food plant e.g. Oc and Pm, and B: non-grasses which include 1) Species which are accepted in the oviposition experiments and in the larval performance experiments such as Cc; 2) Species on which eggs are laid such as Je; 3) Species which are never accepted such as Tr. The food quality of the grass Pm and the non-grass Cc are more or less comparable.

As the pupal weight of *B. anynana* depends on food plant quality and egg size is correlated with female size, the host plant selection of this insect may effect egg size. Egg size differs among individual females. Egg size differences have also been found among lines founded from single females (I.Saccheri, pers. comm.). Since in other insects female fecundity is inversely related to egg size, an increase in fecundity may select for a decrease in egg size in *B. anynana*. However, the female weight of *B. anynana* is positively correlated with the size of the eggs they lay. Wiklund *et al.*, 1987 record that egg size increases with body size among 14 Satyrines adapted to low-temperature environments but not among three species from sunny habitats. When egg size is positively correlated with offspring fitness, selection is expected to favour an increase in egg size. Under laboratory conditions egg size is not correlated with egg hatching or several other life-history traits (Van Oosterhout *et al.*, this volume). Comparable observations made by Wiklund *et al.*, 1987, on the correlation between egg size and offspring-fitness parameters also revealed no clear positive relationship in two Satyrines. To what extent egg size is a direct adaptation to selection pressures rather than a trait that happens to have adaptive significance is thus unclear. According to Reavey (1992) patterns in egg and adult size with respect to feeding specificity and food plant growth form are very similar. The egg size of polyphagous grass feeding Hersperiids tend to be larger than those of specialists (Nakasuji, 1987). In the oligophagous *B. anynana* there may be a selection for larger eggs, related to the feeding ecology of this insect.

B. anynana exhibits phenotypic plasticity. In the wet season their wings have conspicuous eyespots and a white band; in the dry season they are cryptic and lack these pattern elements. Wet season butterflies may use eyespots and white bands as anti-predator devices while dry season insects rest inactively on dead leaves. Laboratory experiments reveal that temperature has a significant effect on the plasticity of the wing pattern (Brakefield & Reitsma, 1991): with an increase of the temperature from 15°C to 28°C the development time decreases and the dry wing season phenotype becomes gradually a wet one. Development time *per se* may be the fundamental factor controlling wing pattern. Therefore, the effect of food quality on developmental period can be expected to have an effect on wing pattern: an increase of development time due to unsuitable food should induce a more dry season-like wing pattern.

Some wing pattern elements (eyespots and band) of small numbers of the butterflies reared on the various food plants were analyzed. The wet season butterflies reared at 28°C on the various food plants showed hardly any differences in wing pattern elements. Perhaps the development time at this temperature of the butterflies grown on

the less suitable food plants is too short to induce dry season pattern characteristics. At 21°C (generally at this temperature "intermediate" butterflies are found) significant differences were found. However, in contrast to the expectation, the specimens grown on the less suitable food plant Pm were "wetter" than those grown on the more suitable Oc.

In general dry season butterflies grown at 17°C have a long developmental period and are large (Windig, in press., Kooi, in press). The temperature relationship between developmental time and size contrasts with that of food plant quality where an increase of developmental period (i.e. poorer quality) is correlated with a decrease of size. This may indicate an interaction between temperature, development and food plant quality. Future work will pay more attention to the effect of food plant, development time and wing pattern.

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REFERENCES

Bernays, E.A. & R. Barbehenn, 1987. Nutritional ecology of grass foliage-chewing insects. In:*Nutritional ecology of insects, mites, spiders and related invertebrates* (F. Slansky Jr. & J.G. Rodriguez eds):147-175. John Wiley & sons, New York etc.

Brakefield, P.M., 1987. Tropical dry and wet season polyphenism in the butterfly Melanitis leda (satyrinae): Phenotypic plasticity and climate correlates. *Biol. J. Linn. Soc.* 31:175-191.

Brakefield, P.M. & N. Reitsma, 1991. Phenotypic plasticity, seasonal climate and the population biology of Bicyclus butterflies (Satyridae) in Malawi. *Ecol. Entomol.* 16:291-303.

Kooi, R.E. Host plant selection by the tropical butterfly Bicyclus anynana (Satyridae). In: *Proceedings 8th International Symposium Insect Plant Relationships*, Wageningen, the Netherlands. (in press).

Nakasuji, F., 1987. Egg size of skippers (Lepidoptera, Hesperiidae) in relation to their host specificity and to leaf toughness of host plants. *Ecol. Res.* 2:175-183.

Reavey, D., 1992. Egg size, first instar larvae and the ecology of Lepidoptera. *J. Zool. Lond.* 227:277-297.

Wiklund, C., 1984. Egg-laying patterns in butterflies in relation to their phenology and the visual apparency and abundance of their host plants. *Oecol.* 63:23-29.

Wiklund, C., B. Karlsson & J. Forsberg, 1987.Adaptive versus constraint explanations for egg-to-body size relationships in two butterfly families. *Am. Nat.* 130:828-838.

Windig, J.J. Seasonal polyphenism in Bicyclus safitza: a continuous reaction norm. *Neth. J. Zool.* (in press).

PHENOTYPIC PLASTICITY AND LIFE HISTORY TRAITS IN SELECTION LINES OF *BICYCLUS ANYNANA*

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Key words: *Bicyclus anynana*, phenotypic plasticity, life history traits, selection experiment

Summary

Two selection lines ('up' and 'down') of the seasonal polyphenic butterfly *Bicyclus anynana* were established at 20°C. Selection was on size of a single hindwing eyespot. Correlated responses occurred in other eyespots and the median band. This study shows that at this intermediate temperature the selection lines had diverged in the direction of the wet season form with eyespots and band ('up') and the dry season form lacking these patterns ('down'). Some, but not all, of the phenotypic divergence was due to differences between the lines in the development time and/or growth rate.

INTRODUCTION

Phenotypic plasticity occurs when a genotype can develop into different phenotypes. If seasonally changing environmental conditions determine the phenotypic expression, and two or more distinct seasonal forms occur, it is called 'seasonal polyphenism' (Shapiro, 1976). The season forms of some African butterflies of the genus *Bicyclus* (Satyrinae) show remarkable differences in wing pattern (Brakefield and Larsen, 1984; Brakefield and Reitsma, 1991). Phenotypes occurring in the wet season (Wsf) exhibit prominent eyespots on their ventral wing surfaces. In contrast, the dry season form (Dsf) has a more or less uniform brown colour. Intermediate phenotypes also occur. The conspicuous wing patterns of the Wsf are thought to function as active anti-predation devices, whereas the cryptic brown coloration of the Dsf mimics the dead leaves on which they rest (Brakefield, 1987). Brakefield and Reitsma (1991) also found that during the wet to dry season succession reproductive activity and the percentage of mature eggs in the ovarioles decreased, while the development of fat bodies increased. The Dsf individuals are thus considered to survive the dry season by inactively resting on the brown leaves, relying on the crypsis of their wings. A larger body size and a longer larval development may maximize stored food resources in the adults. At the onset of the wet season they can immediately begin to reproduce. On the other hand, the Wsf individuals are likely to be favoured by a shorter development time, which enables their progeny to complete development before the biotope dries out. Consequently, the individuals of both seasonal forms are expected to show different trade-offs in the life history traits (see Brakefield, 1987). The transition from wet to dry seasonal forms of *Bicyclus* in Malawi coincides with a decline in temperature (Brakefield

and Reitsma, 1987). Laboratory studies revealed that in a temperature regime of 15 to 28 °C the developmental rate increases, while the wing pattern gradually becomes more like a Wsf phenotype (Brakefield and Reitsma, 1991; Windig, 1992a). Both temperature and development time are envisaged as major proximate causes determining the wing pattern elements.

Selection lines on the size of an eyespot in *Bicyclus anynana* have been established in the laboratory. This experiment describes the phenotypes of one pair of lines ('up' and 'down') for both wing pattern and life history traits. If genetic correlations occur among the wing pattern elements, correlated responses to selection are expected. This study also examines whether correlated responses occur in several life history traits in relation to a number of predictions (see below). Whether a life-history trait affects (or at least is genetically correlated with) a wing pattern element is examined with respect to two criteria. Firstly, does the trait differ significantly between the selection lines, and secondly, within a category of individuals (individuals of the same line and sex), do the same correlations occur between the life history trait and the wing pattern. The following life history traits are analysed: development time, growth rate, egg size, pre-pupal- and adult weight.

Development time is expected to be more rapid for 'up' (Wsf) than for 'down' (Dsf) individuals.

Growth rate is expected to be higher for 'up' than for 'down' individuals. Nylin (1992) reported that plasticity in development time is the most important factor influencing growth rate in the polyphenic species *Polygonia c-album*.

No predictions were made concerning the influence of selection on egg size because Wiklund and Karlsson (1984) were unable to relate this character in other Satyrines to component of fitness.

If the laboratory selection experiment is a good reflection of the seasonal selection, then we expect pre-pupal weight, adult weight and fat content to be higher for 'down' than for 'up' individuals.

MATERIAL AND METHODS

The study material involved two selection lines of *Bicyclus anynana* (Butler) established from one outbred stock at an intermediate temperature of 20 °C. At this temperature the outbred stock produces predominantly intermediate phenotypes. The up-line was selected to produce Wsf-butterflies with large wing eyespots, the down-line produces more or less uniformly brown Dsf-butterflies with small eyespots. The ratio of the width of the 5th. hindwing ventral eyespot to the forewing length was the selected character. Females were allowed to mate at random and the most extreme ($n \geq 30$) selected as parents in each generation for a total of 8 'down' and 9 'up' generations). The caterpillars were reared individually in small net cages on a natural food plant *Oplismenus compositus* (L.) Beauv. at 20 ± 0.5 °C, 80 ± 5% R.H. and 12:12 L:D.

The diameters of freshly laid, individually labelled eggs were measured. This gives a good estimate of weight as in three previous samples of 225 eggs diameter and weight were closely correlated; $r = 0.939$. A total of 204 'down' and 205 'up' eggs were measured. Survival was significantly different between cohorts ($X^2_{(1)} = 8.65$; $P = 0.0033$). Beginning at twenty-one days after egg laying, the surviving larvae were weighed every fifth day and the developmental stage was determined. The pre-pupae were also measured. Emerging butterflies were frozen. The fresh weight of the body without the

wings was measured, together with its dry weight after five days at 50°C. Finally, the fat content was established by adding 2 ml. of 99.8% ether to the insect's body. This treatment was repeated once after two days, before measuring the dry weight after five days at 50°C. The morphometric characteristics of the ventral hind wing were measured following Windig (1992b). The eight variables, representative of the dry and wet forms and/or the sexes, were; wing area, colour, contrast, white band, black ring and white pupil of the 5th. hindwing eyespot, extra ring around the eyespot and marbling.

Multiple ANOVA's (using sex and line) were performed for egg size, pre-pupal weight, adult weight, development time, growth rate and the wing pattern elements. Some of these variables were log transformed to obtain normally distributed variables. The first principal component of the PCA of the wing pattern elements, which gives an overall index of plasticity, was also used in the ANOVA's. Furthermore, significance of the correlation coefficients between various development parameters and wing pattern variables was determined for the sexes and the lines separately.

RESULTS AND DISCUSSION

1.) Wing pattern elements: The 'up' line adult individuals (55♂♂ and 51♀♀) raised in this experiment showed an overall 'wetter' phenotype than the 'down' line individuals (32♂♂ and 44♀♀) (Table 1). Each of the wing pattern variables differed significantly between the lines, while only the relative size of the 5th. hindwing eyespot was selected. Therefore, the unselected wing pattern elements are genetically correlated to the selected wing pattern variables. For a number of wing pattern variables (e.g. 'Black ring') the females showed a more pronounced response to selection than males. Males showed no difference between the lines for the colour of the wings (Fig.1).

Variable	sex	line	sex*line
Wing area	***[1]	*[4]	*
Colour	***[1]	**[3]	**
Contrast	***[1]	***[3]	-
White band; covar. Wing area	-	***[4]	-
Black ring; covar. Wing area	-	***[3]	***
White spot; covar. Wing area	-	***[3]	-
Extra ring; covar. Colour	-	***[3]	-
Marbling; covar. Colour	-	***[4]	-
PC 1	***[2]	***[4]	-
Egg weight	-	**[3]	-
Pre-pupal weight	***[1]	-	*
Dry weight	***[1]	-	-
Fat weight	***[1]	-	-
Development time (Egg to Pre-pupa)	***[1]	**[4]	-
Growth rate (Egg to Pre-pupa)	***[2]	**[3]	-

Table 1. *Significance of F-values in multiple ANOVA for wing pattern variables, first principal component of the wing pattern variables, and life history traits.*
*- $P>0.05$ * $P<0.05$ ** $P<0.01$ *** $P<0.001$.*
[1]Males < Females; [2]Males > Females; [3]Down-line < Up-line; [4]Down-line > Up-line.

2.) Development time and growth rate: Development time and growth rate were highly correlated traits (r=0.877). Both traits varied significantly between the lines (Table 1). This suggests a genetic correlation of these life history traits with the selected wing pattern elements. Table 2 shows that, within lines and sexes, growth rate correlated to a number of wing pattern elements and the PC1. However, only females and not males showed these conspicuous correlations (see also Fig.2). The signs of the correlation coefficients indicate that females with a quicker development and higher growth rate generally emerge as 'wetter' phenotypes. Thus these life history traits may proximately determine some of the phenotypic plasticity in particular wing pattern variables (i.e. Black ring, Extra ring and Contrast). However,if the wing pattern data are corrected for the variance due to differences in development time (or growth rate), highly significant variation remains between the selection lines. This indicates that a substantial response to selection occurred in females independently of development time.

3.) Egg size: Eggs in the 'up' line sample were significantly larger than those of the 'down' line sample (Table 1). Significant weight differences also occurred between the lines at the 21th., 26th. and 31th. day of development (not shown in Table). However, egg size did not correlate with the individual larval weight at these times, nor with development time or any other life-history trait. Egg size was also not correlated with the wing pattern elements within categories of individuals.

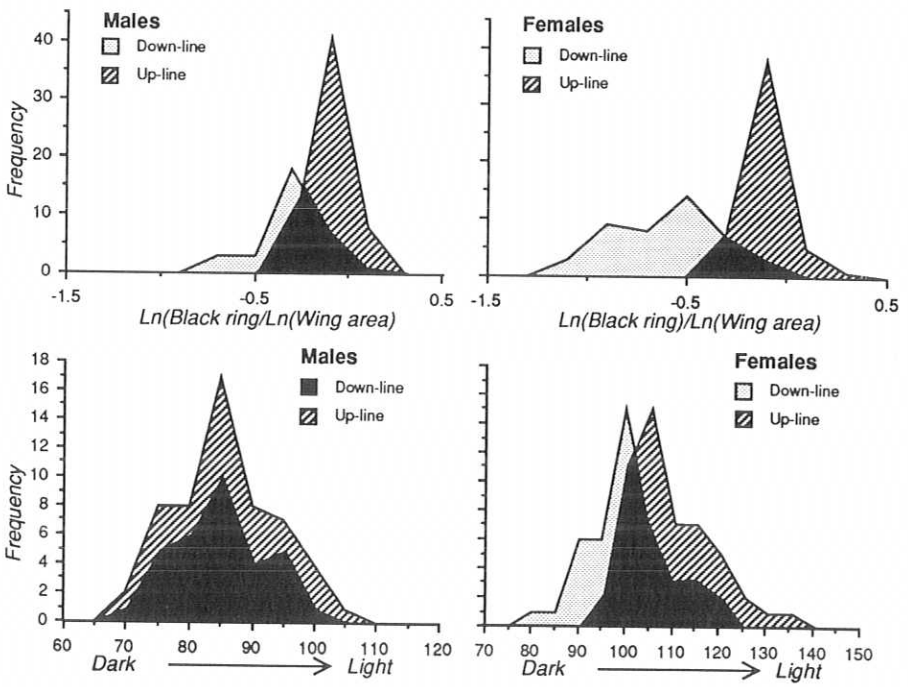

Figure 1. *Frequency distribution of phenotypes for blackring (top) and colour of the wings (bottom); males left, females right.*
Blackring ♂♂: F=106.6 P<0.001; ♀♀: F=147.6 P<0.001
Wing colour ♂♂: F=0.001 P is N.S.; ♀♀ F=19.71 P<0.001

4.) Pre-pupal and adult weight: Final weight did not differ significantly between the lines, even though these differed for development time (Table 1). Significant weight differences only occurred between the sexes; females are larger. Within categories of individuals, the hind wing area was the only wing pattern variable which showed a significant positive correlation with final weight (Table 2).

Wing area	Egg wt	PP wt	Dry wt	Fat wt	Dev 1	Dev 2	GR 1	GR 2
Dn ♂♂	0	+++	+++	++	---	-	+++	+++
Dn ♀♀	0	+++	+++	++	0	0	0	0
Up ♂♂	0	+++	+++	+++	-	0	+++	+++
Up ♀♀	0	+++	+++	+	0	0	+	0

Colour	Egg wt	PP wt	Dry wt	Fat wt	Dev 1	Dev 2	GR 1	GR 2
Dn ♂♂	0	0	0	0	0	++	-	--
Dn ♀♀	0	---	-	0	0	--	0	0
Up ♂♂	0	0	0	0	+	+++	+++	--
Up ♀♀	0	0	0	-	+++	+++	---	---

Contrast	Egg wt	PP wt	Dry wt	Fat wt	Dev 1	Dev 2	GR 1	GR 2
Dn ♂♂	0	0	0	0	0	0	0	0
Dn ♀♀	0	0	+++	0	---	---	+++	+++
Up ♂♂	0	0	0	0	0	0	0	0
Up ♀♀	0	-	0	0	0	---	0	+++

Black ring	Egg wt	PP wt	Dry wt	Fat wt	Dev 1	Dev 2	GR 1	GR 2
Dn ♂♂	0	0	0	0	0	0	0	0
Dn ♀♀	0	0	0	0	---	---	+++	+++
Up ♂♂	0	0	0	0	-	--	++	+
Up ♀♀	0	0	0	0	0	---	0	+

Extra ring	Egg wt	PP wt	Dry wt	Fat wt	Dev 1	Dev 2	GR 1	GR 2
Dn ♂♂	0	0	0	0	0	0	0	0
Dn ♀♀	0	0	0	0	--	---	+	+++
Up ♂♂	0	0	0	0	0	0	0	0
Up ♀♀	0	0	0	0	-	--	++	+++

PC 1	Egg wt	PP wt	Dry wt	Fat wt	Dev 1	Dev 2	GR 1	GR 2
Dn ♂♂	0	0	0	0	0	0	0	0
Dn ♀♀	0	0	0	0	+++	+++	---	---
Up ♂♂	0	-	0	-	0	0	0	0
Up ♀♀	0	0	0	0	0	++	0	--

Table 2. *Significance and sign of correlation coefficients of selected wing pattern variables with life-history traits: egg weight (Egg wt), pre-pupal weight (PP wt), fat weight (Fat wt), the development time egg to pre-pupa and egg to adult (resp. Dev 1 and 2) and the growth rate egg to pre-pupa and egg to adult (resp. GR 1 and 2).*
0 $P>0.1$ +/- $P<0.1$ ++/-- $P<0.05$ +++/--- $P<0.001$

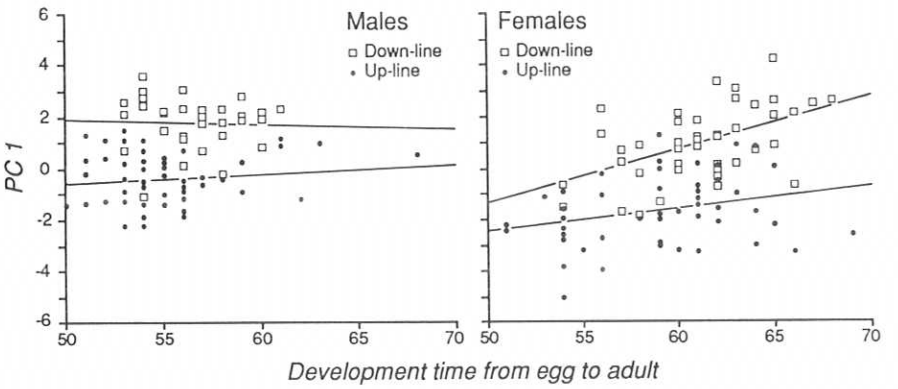

Figure 2. *Development time in days from egg to adult plotted against the first principal component (PC1) for 'Up-line' and 'Down-line ' males (right) and females (left).*
Down-line ♂♂: $r^2=0.002$; P is N.S. Up-line ♂♂: $r^2=0.028$; P is N.S.
Down-line ♀♀: $r^2=0.266$; $P<0.01$ Up-line ♀♀: $r^2=0.085$; $P<0.05$

In summary, selection on a single wing pattern element produces correlated responses in all the measured wing pattern traits. High genetic correlations exist between these traits. Some of the variation across the lines is due to differences in development time and/or growth rate. The majority of the variation, however, is not accounted for by these differences; a direct phenotypic response to selection occurred. A smaller response to selection occurred in some wing pattern elements in males than in females. This may be because selection was only on females. Another explanation may be found in disruptive sexual selection in the field. The fitness of male butterflies depends on the number of successful matings, and therefore at least in part on their flight activity. Flight activity and mobility probably increases for males who more effectively use solar radiation. A dark wing colour may contribute to this, and would therefore be a strongly selected trait for male butterflies. Females, on the other hand, may be more favoured with light coloured wings if this leads to a more effective crypsis. Such a difference in sexual selection may not only account for the difference in selective response in certain wing pattern traits, but also for the difference between the sexes in the extent of the correlation with life history traits.

REFERENCES

Brakefield, P.M., and T.B. Larsen, 1984. The evolutionary significance of dry and wet season forms in some tropical butterflies. *Biol. J. Linn. Soc.* **22**: 1-12.

Brakefield, P.M., 1987. Tropical dry and wet season polyphenism in the butterfly *Melanitis leda* (Satiridae): phenotypic plasticity and climatic correlates. *Biol. J. Linn. Soc.* **31**: 175-191.

Brakefield, P.M., and N. Reitsma, 1991. Phenotypic plasticity, seasonal climate and the population biology of *Bicyclus* butterflies in Malawi. *Ecol. Entom.* **10**: 291-303.

Nylin, S., 1992 (in press). Seasonal plasticity in life history traits: Growth and development in *Polygonia c-album* (Lepidoptera: Nymphalidae). *Biol. J. Linn. Soc.*

Shapiro, A.M., 1976. Seasonal polyphenism. *Evol. Biol.* **9**: 259-333.

Wiklund, C. and B. Karlsson, 1984. Egg size variation in Satyrid butterflies: adaptive vs historical, "Bauplan", and mechanistic explinations. *Oikos* **43**: 391-400.

Windig, J.J., 1992a. Seasonal polyphenism in *Bicyclus safitza*, a continuous reaction norm. *N. J. Zool.* (in press).

Windig, J.J., 1992b. Qualification of the butterfly wing patterns using an image analyser. *J. Res. Lepid.* (in press).

SPATIAL AND TEMPORAL VARIATION IN POPULATIONS OF LIRIOMYZA HUIDOBRENSIS (Diptera, Agromyzidae)

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Key words: *Liriomyza huidobrensis*, introduction, population structure, allozymes

Summary

The population structure of *Liriomyza huidobrensis*, which is recently introduced into Europe, was analysed based on allele frequencies at polymorphic enzyme loci. A positive relation between geographic and genetic distance was observed. F_{ST} values were 0.027 in The Netherlands, indicating a relative high gene flow, and 0.058 in Europe.

INTRODUCTION

The introduction of insect species from one continent into another has often led to severe plagues. Recent examples of such events in Europe are the introductions of *Frankliniella occidentalis* (Pergande), *Bemisia tabaci* (Gennadius), *Liriomyza trifolii* (Burgess) and *Liriomyza huidobrensis* (Blanchard).

Despite the great economic importance of such events, little is known about the mechanisms of successful introductions (Menken & Ulenberg, 1987). For the recent introduction of *Frankliniella occidentalis* into Europe it is stated (Vierbergen, 1987; Robb et al., 1988; Brødsgaard, 1989) that this was preceded by the development of resistance to the major insecticides. The distribution area was subsequently extended by human trade activities: the export and import of infested plant products. For *Liriomyza huidobrensis* also insecticide resistance was reported (Raman, 1988).

Liriomyza huidobrensis was described from Argentina in 1926 and occurs in the greater part of South America. In North America outbreaks are reported since 1938 (Spencer, 1973). The species is intercepted in Europe every now and then for more than a decade (Trouvé et al., 1991). Since 1989 the species is established in The Netherlands (De Goffau, 1991) and later on also the United Kingdom, Germany and France, possibly via The Netherlands (Trouvé et al., 1991).

In Europe *L. huidobrensis* primarily occurs as a pest in glasshouses. However, in the summer it is also frequently found outdoors, causing heavy infestations. In The Netherlands the species is able to survive the winter season outdoors (Van der Linden, this Volume). Although a successful year cycle outdoors can be doubted because of the lack of suitable hostplants early in the year.

Electrophoretic data provide a powerful tool to study population genetic aspects of introductory events and further dispersal (Menken & Ulenberg, 1987). Determination of allozyme frequencies makes it possible to describe the genetic structure of populations

by estimating genetic variability, genetic distance and gene flow between populations.

The present paper describes geographic variation of *L. huidobrensis*. This was studied by measuring alllele frequencies at polymorphic enzyme loci in a number of European populations and one from South America. Furthermore, two Dutch locations were sampled in two subsequent years.

MATERIAL AND METHODS

Table 1 lists the collection data. Most populations were collected in 1991. E-4 and A-1 were collected in 1990 and 1992 respectively. The Dutch locations Enkhuizen-2 and Huissen were sampled in two subsequent years (both years in August/September), (Table 1: N-2/R-2 and N-3/R-3).

Table 1. Collection data.

Location	Year	Code	Location	Year	Code
Enkhuizen-1 (NL)	1991	N-1	Versailles (F)	1991	E-2
Enkhuizen-2 (NL)	1991	N-2	Chambray-les-Tours (F)	1991	E-3
Huissen (NL)	1991	N-3	Zürich (CH)	1990	E-4
Almere (NL)	1991	N-4	Carabayllo (Peru)	1992	A-1
Baarlo (NL)	1991	N-5			
Barendrecht (NL)	1991	N-6	Enkhuizen-2	1992	R-2
Hampshire (UK)	1991	E-1	Huissen	1992	R-3

The number of individuals (all adult females) per population studied for each locus varied between 18 and 28. From a larger number of enzyme loci, that gave good electrophoretic results in *L. huidobrensis* (Oudman, in prep.), 6 polymorphic loci, with known inheritance of the alleles, were chosen for this study: Hexokinase-1 and -2 (*Hk-1* and *Hk-2*), Isocitrate dehydrogenase (*Idh*), 6-Phosphogluconate dehydrogenase (*6Pgdh*), Phosphoglucose isomerase (*Pgi*) and Phosphoglucomutase (*Pgm*).

Cellulose acetate gel electrophoresis was carried out following the procedures of Hebert & Beaton (1989) and Oudman (1992), using Titan III cellulose acetate plates (Helena Laboratories, Beaumont, Texas, USA). Two different buffers were used for electrophoresis: 0.025 M Tris-Glycine pH 8.5 (for *Hk*, *Idh* and *Pgm*) and 0.02 M Phosphate pH 7.0 (for *6Pgdh* and *Pgi*). Gels were run at 200 V (30 V/cm) during 0.3 h.

The genetic structure of populations is described using F-statistics, following the procedures of Weir & Cockerham (1984). In addition Nei's genetic distances (Nei, 1972) between populations were determined. Calculations were performed with the computer programs Thèta (Ellis, 1989) and BIOSYS (Swofford & Selander, 1989).

RESULTS

The frequency distributions of the alleles that were most common in the total population are shown in Table 2. Two categories of enzymes can be distinguished: One consists of *Hk-2*, *6Pgdh* and *Pgm* with relative high frequencies of the most common

allele and fixation in a number of populations. The other category is formed by *Hk-1*, *Idh* and *Pgi*, that had intermediate frequencies of the most common allele and were polymorphic in all populations. The observed heterozygosities per population (Table 2) were in the range from 0.21 to 0.33.

Table 2. Frequencies of the most common alleles (in the total population) and mean heterozygosity per population.

Pop.	*Hk-1*	*Hk-2*	*Idh*	*6Pgdh*	*Pgi*	*Pgm*	$H_{obs.}$	(s.d.)
N-1	0.62	1	0.52	1	0.73	1	0.25	(0.11)
N-2	0.64	1	0.33	1	0.61	0.92	0.24	(0.09)
N-3	0.62	0.98	0.62	0.94	0.58	0.96	0.32	(0.12)
N-4	0.62	0.96	0.44	0.98	0.78	0.86	0.29	(0.08)
N-5	0.74	1	0.71	0.93	0.67	0.95	0.21	(0.08)
N-6	0.68	0.96	0.46	1	0.45	0.98	0.29	(0.11)
E-1	0.54	0.96	0.64	1	0.55	1	0.25	(0.10)
E-2	0.43	0.80	0.73	1	0.60	1	0.33	(0.11)
E-3	0.66	0.86	0.72	1	0.23	1	0.30	(0.10)
E-4	0.60	1	0.74	1	0.68	1	0.24	(0.11)
A-1	0.61	1	0.63	1	0.61	1	0.23	(0.11)
R-2	0.71	1	0.23	1	0.73	0.77	0.26	(0.08)
R-3	0.55	0.86	0.52	1	0.77	1	0.25	(0.10)

Table 3 summarizes the results of F-statistics applied on various groups of populations. The F_{ST} value of the Dutch populations (0.027) was significantly lower than that of the combined European populations (0.058). This indicates that there was less differention between populations within The Netherlands than within the whole of Europe. Including the South American population (A-1) in the calculations did not affect the European F_{ST} significantly (0.051).

Table 3. F_{ST} values for various groups of populations. (The capitals refer to the first letter of the population code, Table 1; n = number of populations)

Population Group	F_{ST}	(s.d.)
A. Netherlands (N, n=6)	0.027	(0.010)
B. Europe (N & E, n=10)	0.058	(0.013)
C. World (N & E & A, n=11)	0.051	(0.012)

Figure 1 presents the relation between Nei's genetic distance and the geographic distance among European populations. The relation between those two distances was: genetic distance = geographic distance * $4.0 * 10^{-5}$ + 0.019 (n=45, r=0.45, $p<0.01$). This indicates that, within Europe, geographically closer populations are also

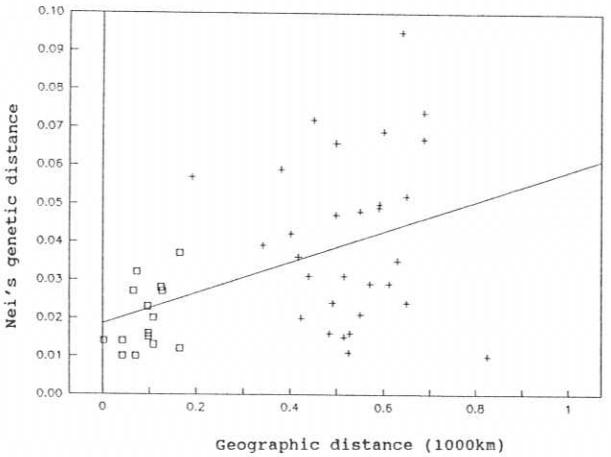

Figure 1. The relation of geographic distance with Nei's genetic distance for European populations of Liriomyza huidobrensis. *(□: within The Netherlands, +: between The Netherlands and the rest of Europe and within the rest of Europe.)*

genetically more identical.

After adding the data of the South American population (A-1) the positive correlation between genetic and geographic distance disappeared (not shown). This can also be inferred from the data in Table 4. This table shows genetic distances of four groups of populations: 1. between populations within The Netherlands, 2. between populations from The Netherlands and from the rest of Europe, 3. between populations within the rest of Europe, and 4. between populations from Europe (including The Netherlands) and the South American population. The mean genetic distance between the European populations and the South American population was much lower than the mean genetic distance within Europe. This indicates that the allele frequencies of the South American population were more or less intermediate within the range of the European populations.

Table 4. Mean values and ranges of Nei's genetic distance between applied on several groups of populations. (The capitals refer to the first letter of the population code, see Table 1 and text; n= number of calculated distances).

Population groups	Nei's genetic distance Mean	(Range)
1. N/N (n=15)	0.020	(0.010 - 0.037)
2. N/E (n=30)	0.041	(0.011 - 0.095)
3. E/E (n=6)	0.043	(0.010 - 0.069)
4. N+E/A (n=10)	0.018	(0.005 - 0.040)

It can also been inferred from Table 4 that the Dutch populations did not have a special place within the European populations. The mean genetic distance between the populations from The Netherlands and those from the rest of Europe (0.041) was not

significantly different from the mean genetic distance between the populations within the rest of Europe (0.043).

Variation in time was studied by sampling the Dutch populations 'Enkhuizen-2' and 'Huissen' in two subsequent years (N-2/R-2 and N-3/R-3). The allele frequencies are shown in Table 2. G-tests performed for each locus revealed no significant differences between the allele frequencies in subsequent years for neither of the two populations. Nei's genetic distances between the samples were 0.013 for N-2/R-2 and 0.018 for N-3/R-3.

DISCUSSION

A number of processes can affect the genetic differentiation between populations of introduced species:
1. Introductions from different origins and founder effects enlarge the differences between populations.
2. Repeated introductions from the same origin and gene flow reduce the differences between populations.

The high genetic similarities between samples of subsequent years from populations in The Netherlands show that *L. huidobrensis* is established in The Netherlands. The positive correlation between genetic and geographical distance (Fig. 1) points to substantial gene flow between populations. The F_{ST} value of 0.027 within The Netherlands also falls in the range of species with good dispersal abilities (McCauley & Eanes, 1987; Menken, 1989, 1990). This level of gene flow is consistent with the observation of a rapid expansion of the *L. huidobrensis* in The Netherlands since the first record in 1989 (De Goffau, 1991).

On a European scale we observed a higher F_{ST} value (0.058) and higher genetic distances between populations (Table 4). This points to the probability of multiple introductions from outside the area studied. If the introduction of *L. huidobrensis* into the other European countries would have taken place via The Netherlands, as is suggested by Trouvé et al. (1991), a relative low mean genetic distance between The Netherlands and the rest of Europe would be expected. This is obviously not the case (Table 4). Moreover, the relative low genetic distance between the European populations and the South American population may point to South America as the source of introduction(s). This is consistent with the regular interceptions of *L. huidobrensis* in plant material exported from South American countries (Trouvé et al., 1991; Internal report Plant Protection Service, The Netherlands). On the other hand the data with respect to South America have to be interpreted cautiously, because only one population was examined, which may not be representative. We have planned a further study on these aspects by the analysis of frequencies of rare alleles.

It is likely that the genetic structure of the populations in Europe will change in course of time. There has been insufficient time since the introduction of the species into Europe for the populations to reach equilibrium with respect to gene flow, genetic drift and selection. Thus the observed F_{ST} values may be related to time since radiation, rather than reflecting actual gene flow levels. In course of time gene flow on a larger scale will reduce F_{ST}. On the other hand genetic drift in isolated populations may enlarge F_{ST}. However, the continued existence of isolated populations is rather unprobable because such populations are easily eradicated by crop protection measures. Therefore,

we expect population differentiation of *L. huidobrensis* in Europe to be reduced in the course of time.

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REFERENCES

BRØDSGAARD, H.E., 1989 - Frankliniella occidentalis (Thysanoptera; Thripidae) - a new pest in Danish glasshouses. A review. *Tidsskr. Planteavl* **93**, 83-91.

DE GOFFAU, L.J.W., 1991 - Liriomyza huidobrensis (Blanchard) (Diptera: Agromyzidae) a new economically important leaf miner in The Netherlands. *Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam* **2**, 41-45.

ELLIS, W.N., 1989 - *Thèta version 1.01*. Institute of Taxonomic Zoology, Amsterdam.

HEBERT, P.D.N. & M.J. BEATON, 1989 - *Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis*. Helena Laboratories, Beaumont.

MCCAULEY, D.E. & W.F. EANES, 1987 - Hierarchical population structure of the milkweed beetle, Tetraopes tetraophthalmus (Foster). *Heredity* **58**, 193-201.

MENKEN, S.B.J., 1989 - Electrophoretic studies on geographic populations, host races, and sibling species of insect pests. In: *Electrophoretic Studies on Agricultural Pests* (eds H.D. Loxdale & J. den Hollander): 181-202. Clarendon Press, Oxford.

MENKEN, S.B.J., 1990 - Genetical population structure in insects. *Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam* **1**, 82-87.

MENKEN, S.B.J. & S.A. ULENBERG, 1987 - Biochemical characters in agricultural entomology. *Agr.Zool.Rev.* **2**, 305-360.

NEI, M., 1972 - Genetic distance between populations. *Am. Nat.* **106**, 283-292.

OUDMAN, L., 1992 - Identification of economically important Liriomyza species (Diptera, Agromyzidae) and their parasitoids using enzyme electrophoresis. *Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam* **3**, 135-139.

RAMAN, K.V., 1988 - Integrated pest management for potatoes in developing countries. *CIP Circular, International Potato Center* **16**, 1-8.

ROBB, K.L., J.P. NEWMAN & M.P. PARRELLA, 1988 - The biology and control of the western flower thrips. *TSWV Newsletter* **3**, 6-12.

SPENCER, K.A., 1973 - *Agromyzidae (Diptera) of Economic Importance. Series Entomologica 9*. Junk, The Hague.

SWOFFORD, D.L. & R.B. SELANDER, 1989. *BIOSYS-1*. Illinois Natural History Survey.

TROUVÉ, C., M. MARTINEZ, M. PHALIP & C. MARTIN, 1991 - Un nouveau ravageur en Europe. La mouche mineuse sud-américaine. *Phytoma* **429**, 42-46.

VIERBERGEN, B., 1992 - Interceptions of species of the genus Frankliniella in the Netherlands (Thysanoptera: Thripidae). *Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam* **3**, 175-180.

WEIR, B.S. & C.C. COCKERHAM, 1984 - Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

THE TRANSMISSION OF LYME BORRELIOSIS IN THE NETHERLANDS

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Key words: *Ixodes ricinus*, *Borrelia burgdorferi*, Lyme disease

Summary
Sheep ticks, *Ixodes ricinus*, infected with *Borrelia burgdorferi*, the causative agent of Lyme disease, are commonly found in every province of The Netherlands. The woodmouse, *Apodemus sylvaticus*, is at least locally a reservoir host for *Borrelia*, but several other wild vertebrates may also be involved.

INTRODUCTION

The bacterial agent causing Lyme-borreliosis or Lyme disease, was discovered in the internal organs of the North American tick, *Ixodes dammini*, as recently as 1982. Two years later it was described as a new species in the genus *Borrelia* (Spirochaetaceae) by Johnson et al. (1984) who named it *Borrelia burgdorferi* after its discoverer Willy Burgdorfer. Soon it was found out that the organism infects many species of wild mammals and birds all over the northern hemisphere. The infection is passed on from one animal to another through parasitizing ticks belonging to the *Ixodes ricinus*-complex. Incidentally an infected tick may attach to the skin of a human victim and transmit the infection. This happens quite often judging by the numbers of people carrying serum antibodies to *B. burgdorferi*. Of a random sample of 1052 Dutch blood donors 9 % revealed anti-*Borrelia* immunoglobulins (Nohlmans et al., 1991). Among people who habitually enter the biotope of *Ixodes ricinus* in The Netherlands, such as hunters or forestry workers, the seroprevalence is considerably higher (15% and 20% respectively, Nohlmans et al., 1991, Nauta et al. 1991). Most of these *Borrelia* infections remain symptomless. In only a minority of cases a skin rash develops, gradually expanding from the site of the tick bite. This so called Erythema Chronicum Migrans (ECM or EM) is the earliest clinical manifestation of Lyme disease. Later manifestations occur less frequently but are much more serious. Such symptoms involve the central nervous system, the joints of limbs or the heart muscle.

VECTOR TICKS OF *BORRELIA BURGDORFERI*

A number of tick species, belonging to different genera (*Ixodes*, *Dermacentor* and *Amblyomma*) have been tested in order to evaluate their ability to transmit *B. burgdorferi*. *I. ricinus*, *Ixodes dammini*, *Ixodes scapularis* and *Ixodes pacificus* proved to be competent vectors (Mather et al. 1990a, Monin et al. 1989, Piesman & Sinsky, 1988, Piesman, 1989). There is strong evidence that *Ixodes persulcatus*, another member of the *I. ricinus*-complex, is also a vector (Al et al. 1990, Kawabata et al., 1987). Together the range of these vectors covers most of the temperate climatic zone of the

northern hemisphere. Indeed, cases of Lyme disease are being reported from Western Europe through Japan and in wide areas of the USA (Schmid, 1985, Korenberg, 1986). In The Netherlands the sheep tick *I.ricinus* is the only species of tick that regularly parasitizes man (van Bronswijk, 1979). Most populations of sheep ticks in The Netherlands are infected with *B. burgdorferi*. Like most members of the Ixodidae, *I. ricinus* is a three-host tick.The life cycle of these species follows a strict pattern: EGG-*eclosion* - LARVA (first blood meal on host 1) - *moult* - NYMPH (second blood meal on host 2) - *moult* - ADULT (copulation and third blood meal on host 3) - *oviposition*
Usually only a small percentage (1 - 3 %) of the unfed larvae is infected with *Borrelia* (Aeschlimann, 1986, Levine et al., 1985, Miserez, 1990, Monin et al., 1989, Lindsey et al., 1991, Piesman, 1991, Piesman et al., 1986). These infections must be due to transovarial transmission of the organism from the female tick to its offspring. Probably, many more ticks acquire an infection when they take their first blood meal as a larva (Donahue et al. 1987, Levine et al., 1985, Mather et al., 1989, Mather et al., 1990b). Thus 9 - 15% of unengorged nymphs collected in Veldhoven (51.24 N, 5.24 E), Gaasterland (52.54 N, 5.35 E), and the island of Texel (53.03 N, 4.47 E) were infected with spirochetes, most likely *B. burgdorferi* (de Boer et al., in press). The infection rate of unfed adults is generally higher still (de Boer et al., in press, Hubalek et al., 1991, Kahl & Knülle, 1988b, Maupin, 1991). This reflects the fact that the adults have had two bloodmeals i.e. two chances to acquire an infection instead of only one.

In spite of the higher infection rate of the adults, nymphs are believed to be responsible for the majority of *Borrelia* infections in man. The number of nymphs present on the vegetations is usually much higher than the number of adults. It may also be significant that an adult tick is more readily discovered and removed than a nymph. Even if the tick is already attached to the skin, prompt removal reduces the chance of infection (Piesman et al. 1987, Piesman et al. 1991).

THE OCCURRENCE OF *IXODES RICINUS*

Availability of hosts, especially hosts for the adults, must be an important limiting factor for the occurrence of ticks. Adult sheep ticks require a relatively large animal to feed on. Animals smaller than a stoat (*Mustela erminea*) are never infested with adults of *I. ricinus* (Arthur, 1963). We can only speculate about an ecological explanation for this. The adults need a host not only to feed on but also to find a mate. Larger animals accumulate more ticks and consequently offer a better chance to encounter a member of the opposite sex. In addition to this, the amount of blood ingested by the adult ticks may endanger the survival of a small host. A single *I. ricinus* female takes an equivalent of 0.6 ml of blood (Arthur, 1962). For a mouse this would be an heavy loss.
There is strong evidence that the occurrence of *I. dammini* in the eastern USA is determined largely by the availability of white-tailed deer (*Odocoileus virinianus*) as hosts for the adult ticks (Anderson et al. 1987, Fish & Dowler, 1989, Matuschka et al., 1986, Wilson et al., 1990a, Wilson et al., 1990b). This dependence on deer cannot be so strict for *I. ricinus*. In at least two forest areas in The Netherlands, one on Texel and one near Heiloo (52.36 N, 4.43 E), *I. ricinus* is very numerous whereas deer or animals of comparable size are not available as hosts (de Boer, 1991, Broekhuizen et al., 1992). Medium sized mammals like hedgehogs or hares may serve as hosts for the adult ticks here (Milne, 1949a&b, Pretzmann et al. 1964). Rabbits are also present, sometimes in great numbers. There is evidence, however, that these animals are poor hosts for *I. ricinus* (Milne, 1949a&b). This may be connected with the immunological intolerance for tick bites that readily develops in a rabbit (Brossard et al. 1990). An investigation into the role of rabbits as hosts for adult *I. ricinus* is needed.
At least one abiotic factor is decisive for the occurrence of *I. ricinus*. This concerns the humidity conditions at the ground level. A sheep tick spends more than 95% of its time away from a host on the vegetation and in the layer of litter on the ground. Loss of body water through evaporation is a constant threat during that time. The water balance is

maintained by extracting water vapour from the surrounding air. This can be accomplished only if the atmospheric humidity is at least 80% RH (Kahl & Knülle, 1988a). Such conditions can be found near the ground in shady forests, but sometimes also in more open vegetations. In The Netherlands deciduous and mixed forests are a very important if not the most important habitat for *I. ricinus*.

RESERVOIR HOSTS OF *BORRELIA BURGDORFERI*

Experimentally various species of rodents can be infected with *B. burgdorferi*. The animals become infectious for the ticks that are fed on them and remain so for several months (Burgdorfer et al., 1987, Donahue et al. 1987). Wild birds have also been shown to infect the ticks that were feeding on them (Weisbrod et al. 1989). Clearly, the adaptation of *B. burgdorferi* is not restricted to one or a few species of reservoir hosts. The reservoir hosts of *B. burgdorferi* in The Netherlands must be sought among the vertebrate species that are regularly parasitized by *I. ricinus*. The list of species that have been recorded as hosts of *I. ricinus* is very long (Arthur, 1963). However, the contribution of many of these species in feeding ticks may be unimportant in a quantitative sense. In a forest near Hannover, Germany, Walter and Liebish (1980) recorded great differences in the intensity of the infestation of the various birds and mammals. When their data are combined with information on the population density of the vertebrate species, an 'educated guess' about the relative importance of the different host species, can be made. The number of larvae fed by mammals is certainly much greater then the number fed by birds. For nymphs the difference between birds and mammals is not so great. The mammal species that probably play a dominant role as hosts for immature *I. ricinus* are: hedgehog (*Erinaceus europeus*), shrew (*Sorex araneus / S. coronatus*), bank vole, woodmouse and perhaps the squirrel (*Sciurus vulgaris*). Shrews serve as hosts for larvae but not for nymphs. There is evidence from several studies that voles, in particular the bank vole, are less attractive or less suitable for immature sheep ticks than for instance the *Apodemus* species or the hedgehog (Labuda et al., 1991, Matuschka et al. 1990a, 1990b & 1991, Perez-Eid, 1990). However, in one study (Steele, 1984) it was found that a vole (*Microtus agrestis*) was more attractive than the woodmouse. Perhaps host preference is a population character rather than a species character.

Again there is great uncertainty about the role of rabbits. More information is also needed concerning the role of hares (*Lepus europeus*), roe deer (*Capreolus capreolus*), polecats (*Putorius putorius*) and foxes (*Vulpes vulpes*). Among birds the blackbird (*Turdus merula*), the songthrush (*Turdus philomelos*) and the jay (*Garrulus glandarius*) appear to be relatively important. But also wrens (*Troglodytes troglodytes*), robins, dunnocks (*Prunella modularis*) and tits (*Parus spec.*) are frequently parasitized by *I. ricinus*. There is evidence that the dunnock becomes intolerant for tick bites on frequent exposure and this may be true of other birds as well (Walter, 1987).

It is only possible to compare and combine the infestation rates found in different studies if the infestation of one species is measured *relative to* the infestation of at least one other species, sampled at the same time in the same locality. For western Europe the woodmouse (*Apodemus sylvaticus*) could be a suitable reference species.

Even less information is available concerning the infection of the various vertebrates with *B. burgdorferi*. Of the European vertebrates the yellow necked mouse (*Apodemus flavicollis*), the woodmouse (*Apodemus sylvaticus*), the bank vole (*Clethrionomys glareolus*) and the robin (*Erithacus rubecula*) have been shown to infect parasitizing sheep ticks with spirochetes, probably *B. burgdorferi* (Aeschlimann, 1986, de Boer et al., in press, Hovmark et al., 1988, Humair et al., 1990). On the island of Texel, in Gaasterland and in Veldhoven woodmice were captured and subjected to xenodiagnosis, viz. larvae of *I. ricinus* were placed on the animal, which is subsequently confined in a cage with a grid bottom. The cage is then positioned over a water-filled tray so that the engorged ticks can be easily collected when they drop off. After the ticks moulted to

nymphs their internal organs were examined for spirochetes. The percentage of mice that were infected with spirochetes was 47% (n=45), 29% (n=58) and 0% (n=64) for the three locations respectively (de Boer et al., in press). The absence of spirochete infected mice in Veldhoven is interesting. One bank vole out of 14 captured in the same area at the same time, proved to be infected with tick-transmitted spirochetes. Also, spirochete infected *I. ricinus* nymphs (up to 11%) were collected in the same location during the subsequent spring. A much more detailed study would be required to explain these findings. However, it is clear that, at least locally, the woodmouse is a reservoir of tick-transmitted spirochetes, most likely *B. burgdorferi*. The role of other vertebrate species that are frequently parasitized by *I. ricinus* still has to be investigated.

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REFERENCES

AL, C., R. HU, K.E. HYLAND, Y. WEN, Y. ZHANG, Q. QUI, D. LI, X. LIU, Z. SHI, J. ZHAO & D. CHENG, 1990. Epidemiological and aetiological evidence for transmisson of Lyme disease by adult Ixodes persulcatus in an endemic area in China. *Int.J.Epidemiol.* 19: 1061-1065.

AESCHLIMANN, A., E. CHAMOT, F. GIGON, J-P. JEANNERET, D. KESSELER & C. WALTHER, 1986. B. burgdorferi in Switzerland. *Zbl.Bakt.Hyg.* A 263: 450-458.

ANDERSON J.F., R.C. JOHNSON, L.A. MAGNARELLI, F.W. HYDE & J.E.MYERS, 1987. Prevalence of Borrelia burgdorferi and Babesia microti in mice on islands inhabited by white-tailed deer. *Appl.Env.Microbiology.* 53: 892-894.

ARTHUR, D.R., 1962. *Ticks and disease*; Pergamon press, Oxford; 445 pp.

ARTHUR, D.R., 1963. *British ticks.* Butterworths; London; 213 pp.

BRONSWIJK, J.E.M.H. VAN, R.H. RIJNTJES, A.F.M. GARBEN, H. VOS, 1979. *De teken van de Benelux-landen*; Koninklijke Nederlandse Natuurhistorische Vereniging; Hoogwoud. 36 pp.

BURGDORFER, W., K.L. GAGE, 1987. Susceptibility of the Hispid Cotton Rat (Sigmodon hispidus) to the Lyme disease spirochete. *Am.J.Trop.Med.Hyg.* 37: 624-8.

BOER, R. DE, 1991. *De transmissie van Lyme borreliose in Nederland.* Praeventiefonds-project nr 28-1791. Eindverslag.

BOER, R. DE, K.E. HOVIUS, M.K.E. NOHLMANS & J.S. GRAY, 1993. The woodmouse (Apodemus sylvaticus) as a reservoir of tick-transmitted spirochetes (Borrelia burgdorferi) in The Netherlands. *Int.J.Med.Microbiol.Parasitol.Inf.Dis.* in press.

BROEKHUIZEN, S., B. HOEKSTRA, V. VAN LAAR, C. SMEENK, J.B.M. THISSEN, 1992. *Atlas van Nederlandse zoogdieren.* Stichting Uitgeverij Koninklijke Nederlandse Natuurhistorische Veriniging. 336 pp.

BROSSARD, M. & V. PAPATHEODOROU, 1990. Immunity against female Ixodes ricinus L.: effect on feeding and haemaglobin digestion. *Ann.Parasitol.Hum.Comp.* 65: 32-36.

DONAHUE, J.G., J. PIESMAN & A. SPIELMAN, 1987. Reservoir competence of white-footed mice for Lyme disease spirochetes, *Am.J.Trop.Med.Hyg.* 36: 92-96.

FISH, D. & R.C. DOWLER, 1989. Host associations of ticks parasitizing medium-sized mammals in a Lyme disease endemic area of Southern New York. *J.Med.Entomol.* 26: 200-209.

HOVMARK, A., T.G.T. JAENSON, E. ASBRINK, A. FORSMAN & E. JANSSON, 1988. First isolations of Borrelia burgdorferi from rodents collected in Northern Europe. *APMIS* 96: 917-920

HUBALEK, Z., J. HALOUZKA & Z. JURICOVA, 1991. A comparison of the occurrence of Borreliae in nymphal and adult Ixodes ricinus ticks. *Zbl.Bakt.* 275: 133-137.

HUMAIR, P.F., N. VITTOZ, M. SIEGENTHALER, A. AESCHLIMANN & L. GERN, 1990. Mammalian and avian reservoirs for Borrelia burgdorferi in a Lyme borreliosis focus in Switzerland. *VII International Congress of Acarology, Abstracts* p. 97.

JOHNSON, R.C., G.P. SCHMID, F.W. HYDE, A.G. STEIGERWALT & D.J. BRENNER, 1984. *Borrelia burgdorferi* sp. nov.: ethiological agent of Lyme disease. *Int.J.Syst.Bacteriol.* 34: 496-497.

KAHL, O. & W. KNÜLLE, 1988a. Water vapour uptake from subsaturated atmospheres by engorged immature ixodid ticks. Exp. Appl. Acarology. 4: 73-83.

KAHL, O. & W. KNÜLLE, 1988b. Wirtssuchaktivität der Schildzecke Ixodes ricinus und ihre Durchseuchung mit Lyme-spirochäten. *Mitt.Dtsch.Ges.Allg.Angew.Ent.* 6: 223-225.

KAWABATA M., S. BABA, K. IGUCHI, N YUMAGUTI & H. RUSSELL, 1987. Lyme disease in Japan and its possible incriminated tick vector, Ixodes persulcatus. *J.infec.Dis.* 156: 854.

KORENBERG, E.I., V.N. KUYUCHECHNIKOV, Y.V. ANANYINA, Y.G. CHERUKHA, 1986. Prerequisites of the existence of Lyme disease in the U.S.S.R. *Zbl .Bakt .Hyg.* A263: 471-472.

LABUDA, M., J. LYSY & O. KOZUCH, 1991. in: F. Dusbabek & V. Bukva (Eds.): Modern Acarology, Academia, Prague and SPB Academic Publishing bv, The Hague, Vol. 2: 29-33.

LEVINE J.F., M.L. WILSON, A. SPIELMAN,1985. Mice as reservoirs of the Lyme disease spirochete. *Am.J.Trop.Med.Hyg.* 34: 355-60.

LINDSAY, L.R., I.K. BARKER, G.A. SURGEONER, S.A. MCEWEN, L.A. ELLIOTT & J. KOLAR, 1991. Apparent incompetence of Dermacentor variabilis and fleas as vectors of Borrelia burgdorferi in an Ixodes dammini endemic area of Ontario. *J.Med.Entomol.* 28: 750-753.

MATHER, T.N., M.L. WILSON, S.I. MOORE, J.M.C. RIBEIRO & A. SPIELMAN, 1989. Comparing the ralative potential of rodents as reservoirs of the Lyme disease spirochete. *Am.J.Epidemiol.*, 130: 143-150.

MATHER, T.N. & M.E. MATHER, 1990. Intrinsic competence of three Ixodod ticks as vectors of the Lyme disease spirochete. *J.Med.Entomol.* 27: 646-650.

MATHER, T.N., S.R. TELFORD III, S.I. MOORE AND A. SPIELMAN, 1990b. Borrelia burgdorferi and Babesia microti: Efficiency of transmission from reservoirs to vector ticks. *Exp.Parasitol.* 70: 55-61.

MATUSCHKA , F.R., D. RICHTER, P. FISCHER & A. SPIELMAN, 1990a. Time of repletion of subadult Ixodes ricinus ticks feeding on diverse hosts. *Parasitol.Res.* 76: 540-544.

MATUSCHKA , F.R., R. LANGE, A. SPIELMAN, D. RICHTER & P. FISCHER, 1990b. Subadult Ixodes ricinus on rodents in Berlin, West Germany. *J.Med.Entomol.* 27: 385-390.

MATUSCHKA, F.R. , P. FISCHER, K. MUSGRAVE, D. RICHTER & A. SPIELMAN, 1991. Hosts on which nymphal Ixodes ricinus most abundantly feed. *Am.J.Trop.* 44: 100-107.

MATUSCHKA F.R., A. SPIELMAN, 1986. The emergence of Lyme disease in a changing enviroment in North America and central Europe. *Exp. Appl. Acarology.* 2: 337-53.

MAUPIN, G.O., D. FISH, J. ZULTOWSKY, E.G. CAMPOS & J. PIESMAN, 1991. Landscape ecology of Lyme disease in a residential area of Westchester County, N.Y., *Am.J.Epidemiol.* 133: 1105-1113.

MONIN, R., L. GERN & A. AESCHLIMANN, 1989. A study of the different modes of transmission of Borrelia burgdorferi by Ixodes ricinus. *Zbl. Bakt.* Suppl 18: 14-20.

MILNE, A., 1949a. The ecology of the sheep tick, Ixodes ricinus. Part I. *Parasitology*, 39: 168-172.

MILNE, A., 1949b. The ecology of the sheep tick, Ixodes ricinus. Part II. *Parasitology*, 39: 173-197.

MISEREZ, V., L. GERN & A. AESCHLIMANN, 1990. Borrelia burgdorferi in ticks of the canton Tessin (Switzerland). *Parasitologia.* 32: 293-299.

NOHLMANS, M.K.E., R. DE BOER, A.E.J.M. VAN DEN BOGAARD, A.A.M. BLAUW & C.P.A. VAN BOVEN, 1990. Voorkomen van Borrelia burgdorferi in Ixodes ricinus in Nederland. *Ned. Tijdschr. Geneedk.* 134: 1300-1303.

NOHLMANS M.K.E., A.E.J.M. VAN DEN BOGAARD, A.A.M. BLAUW & C.P.A. VAN BOVEN, 1991. Prevalentie van Lyme borreliose in Nederland. *Ned. Tijdschr.Geneesk.* 135: 2288-2292.

NAUTA , A.P., H. KUIPER, H. HOUWELING, B.M. DE JONGH, L. WIESSING, L. SPANJAARD, L. SCHAAP, 1991. Lyme borreliose en bosarbeid. *Tijdschr.Soc.Gezondheidsz.* 69: 397-400

PEREZ-EID, C., 1990. Les relations tiques-petit mammifières dans le foyer Alsacien d'encéphalite à tique. *Acarologia* 31: 131-141.

PIESMAN, J., 1989. Transmission of Lyme disease spirochetes. *Exp.Appl.Acarol.* 7: 71-80.

PIESMAN, J., 1991. Experimental acquisition of the Lyme disease spirochete, Borrelia burgdorferi, by larval Ixodes dammini during partial blood meals. *J.Med.Entomol.* 28: 259-262.

PIESMAN, J., J.G. DONAHUE, T.N. MATHER & A. SPIELMAN, 1986. Transovarially acquired Lyme disease spirochetes (Borrelia burgdorferi) in field-collected larval Ixodes dammini. *J.Med.Entomol.* 23: 219.

PIESMAN, J., T.N. MATHER, R.J. SINSKY & A. SPIELMAN, 1987. Duration of tick attachment and Borrelia burgdorferi transmission. *J.Clin.Microbiol.*, 25: 557-558.

PIESMAN, J., G.O. MAUPIN, E.G. CAMPOS & C.M. HAPP, 1991. Duration of adult female Ixodes dammini attachment and transmission of Borrelia burgdorferi, with descripion of needle aspiration method. *J.Inf.Dis.* 163: 895-897

PIESMAN, J., R.J. SINSKY, 1988. Ability of Ixodes scapularis, Dermacentor variabilis and Amblyomma americanum to acquire, maintain and transmit Lyme disease spirochetes. *J.Med.Entomol.* 25: 336-339

PRETZMANN, G., A. RADDA & J. LOEW, 1964. Studien zur Ökologie van Ixodes ricinus. *Z.Morph.Ökol.Tiere.* 54: 393-413.

SCHMID, G.P., 1985. The global distribution of Lyme disease. *Rev.Inf.Dis.* 7: 41-50

STEEL, G.M., 1984. A comparative ecological study of methods for the control of sheep ticks (Ixodes ricinus). 168 pp. D.Phil. thesis, Oxford Univ.

WALTER, G., 1987. Zur Bedeutung der Heckenbraunelle (Prunella modularis) als Wirt des Holzbocks Ixodes ricinus. *Braunschw.Naturk.Schr.* 2: 719-723.

WALTER, G. & A. LIEBISH, 1980. Untersuchungen zur Biologie und Verbreitung von Zecken in Norddeutschland. *Z.angew.Zoologie*, 67: 449-476.

WEISBROD, A.R. & R.C. JOHNSON, 1989. Lyme disease and migrating birds in the Saint Croix River Valley. *App.Env.Microbiol.* 55: 1921-1924.

WILSON, M.L., T.S. LITWIN, T.A.GAVIN, M.C. CAPKANIS, D.C. MACLEAN & A. SPIELMAN, 1990. Host-dependent differences in feeding and reproduction of Ixodes dammini. *J.Med.Entomol.* 27: 945-954.

WILSON, M.L., A.M. DUCEY, T.S. LITWIN, T.A. GAVIN & A. SPIELMAN, 1990. Microgeographic distribution of immature Ixodes dammini ticks correlated with that of deer. *Med.Vet.Entomol.* 4: 151-159.

CONTROL OF THE BLACK VINE WEEVIL (*Otiorhynchus sulcatus*) WITH DIFFERENT ISOLATES OF *HETERORHABDITIS* SP. AND *METARHIZIUM ANISOPLIAE* IN NURSERY STOCK

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Key words: *Otiorhynchus sulcatus*, *Heterorhabditis* sp., *Metarhizium anisopliae*, biological control, nursery stock.

Summary

Three of the five isolates of the insect parasitic nematode *Heterorhabditis* sp. and the insect pathogenic fungus *Metarhizium anisopliae* gave good control of the larvae of the black vine weevil (*Otiorhynchus sulcatus*) in containers outdoors in autumn. In the open ground two of the three isolates of *Heterorhabditis* sp. tested were effective and so was the fungus *M. anisopliae*. All the agents tested were generally less effective in the open ground than in containers outdoors. The results of the experiments indicate that the efficacy of *Heterorhabditis* sp. against the black vine weevil is determined by soil temperature and antagonism in soil.

INTRODUCTION

The black vine weevil (*Otiorhynchus sulcatus*) is a major pest in nursery stock. The larvae damage the roots of many plants and are difficult to control. The weevils are present from June until October and lay eggs from July until September. Hatched larvae overwinter in soil and continue to feed on plant roots. In winter large larvae start to feed on the main roots and the root collar of the plants, causing economically important damage. The only permitted chemical control agent, carbofuran, is not always effective and is toxic to many other organisms, and it is unlikely that better chemicals will become available at least in the short term. However, biological control with parasitic nematodes and pathogenic fungi is an option. Nematodes have proved most successful in greenhouses (Georgis & Poinar, 1984; Klingler, 1988). Only a few applications succeed outdoors. Most success is achieved after applying the nematodes in spring or summer, when soil temperature does not limit the infection proces (Dolmans, 1983; Dorschner et al., 1989; Shanks & Agudelo-Silva, 1990). Outdoor application in autumn, as late as possible, will be most effective to reduce the larval population. Weevils have then stopped laying eggs, most larvae are big enough to be successfully infected by the nematodes and the plants have not yet suffered economically damage. The problem with late applications of nematodes outdoors is the low soil temperature, which reduces the efficacy of many isolates.

In this study six isolates of nematodes were tested for their efficacy outdoors in autumn to control the larvae of the black vine weevil. Preliminary attempts were

also made to clarify the relation between temperature demands and efficacy under different conditions (this includes a climate room experiment which examined the effect of temperature). As well as the nematodes a commercial formulation was tested of the fungus *Metarhizium anisopliae*, which is pathogenic to insects.

MATERIAL AND METHODS

Six nematode isolates of different origins and one isolate of the fungus *Metarhizium anisopliae* were used (Table 1). Granules of dried mycelium of the fungus that sporulate after being mixed through soil were used (Andersch et al., 1990; Storey et al., 1990).

The trials were carried out in containers outdoors, in the open ground and in climate rooms. All experiments were done in Boskoop, the Netherlands. The temperature of the soil in the pots and in the open ground was measured every two hours.

The substrate used in pots consisted of 55% peat pellets, 40% sphagnum-moss peat and 5% aeolian sand. The soil in the open ground experiment contained 34% organic matter and 9% clay fraction (clay peat soil). The test plant was *Thuja occidentalis* 'Brabant'.

The data were statistically processed using ANOVA. In order to be able to analyse the number of larvae the square roots of the data were used.

Table 1 - Biological agents used in the experiments

nematode/fungus	origin	code	experiment*
Heterorhabditis megidis	United Kingdom	UK-H-211	I,II,III
Heterorhabditis megidis	Germany	D-H-HSH	I,II,III
Heterorhabditis megidis	Germany	D-H-HD	III
Heterorhabditis megidis	Netherlands	Nl-H-HF85	I
Heterorhabditis bacteriophora	Italy	I-H-?#	I,II
Heterorhabditis bacteriophora	Australia	Au-H-?#	I
Metarhizium anisopliae	Germany	BIO1020	I,II

 # code number unknown
** I = outdoor container experiment; II = open ground experiment; III = climate room experiment*

I - Outdoor container experiment

There were eight treatments (Table 1), including "untreated" and a treatment with carbofuran; in each treatment 32 plants (8 plants per block, 4 blocks) were used. On 13 May 1991 the test plants were potted up into one-litre containers and placed on the container beds. The substrate of the treatment with *Metarhizium anisopliae* had been mixed with the fungal granules (1 $g.l^{-1}$ soil) on 25 April 1991 and then, without further watering, had been covered and put aside in a warm place (20°C) until potting day on 13 May. The plants were inoculated three times with 15

weevil eggs per plant (on 29 July, 12 August and 27 August 1991) to mimic the seasonal spread of egg-laying of the weevil through the season. On 25 July and 29 November 1991 soil samples of the treatment with *M. anisopliae* were taken and spore density in the soil was determined (Hartwig and Oehmig, 1992). In the carbofuran treatment (37.5 $l.ha^{-1}$, 20% a.i.) there were two applications: on 22 July 1991 and 3 September 1991. The nematodes (15 000 l^{-1} soil) were inoculated on 26 September 1991 and again on 24 October 1991. The plants were harvested at the end of November. The number of live larvae were counted.

II - Open ground experiment

Six treatments were carried out in triplicate (Table 1), including "untreated" and a treatment with carbofuran, using five test plants per replicate, surrounded by 12 border plants. Every replicate of a treatment, including the border plants, was planted on one square metre. The test plants were planted in the open ground on 15 May. On 25 April 15 litres of peaty soil was mixed with 300 grams of *M. anisopliae* granules and pre-incubated as in the container experiment. On 15 May 1991 five litres of this mixture were mixed through the upper 10 centimetres of soil of each replicate (1 m^2) of the *Metarhizium* treatment. The plants were inoculated three times (on 29 July, 12 August and 27 August 1991) with 50 weevil eggs per plant. On 25 July 1991 and 24 March 1992 soil samples of the *Metarhizium* treatment were analysed for spore density. The treatments with carbofuran and the nematodes were done in the same way and on the same days as in the container experiment. However, the nematode dosage was different: $10^6.m^{-2}$. The larvae were counted at the end of March 1992.

III - Climate room experiment

Four treatments (Table 1), including "untreated", at three temperatures were carried out in fourfold, using four test plants per replicate. Plants in one-litre containers were inoculated with 30 eggs per plant and incubated at 20°C in a climate room for two months. On 14 January 1991 the plants were divided into three series and transferred to climate rooms at 9, 12 and 15°C respectively. On 16 January 1991 each pot was inoculated with 15 000 nematodes and placed on a saucer to prevent the nematodes from migrating to other plants. On 26 February 1991 all plants were checked for the presence of live larvae.

RESULTS

The results of the three experiments are presented in table 2. They show that in the outdoor container experiment (I) the nematode isolates H211 and HF85 gave almost complete control of the larvae of the black vine weevil. The result is as good as that achieved with carbofuran. The control results with *M. anisopliae* and the nematode isolate HSH were slightly less successful but still close to 90%. The soil in the pots with *M. anisopliae* contained $6.0x10^7$ spores per gram dry soil in July and $2.0x10^7$ spores per gram dry soil in November. The Italian isolate HI and the Australian isolate HAu did not achieve results as good as those of the other isolates, but nevertheless reduced the number of larvae by up to 60 to 70% compared with "untreated".

In the open ground (II) neither carbofuran nor the nematode isolate HSH reduced the number of larvae statistically significantly. Compared with "untreated" the fungus *M. anisopliae* and the nematode isolates H211 and HI statistically significantly controlled the larvae. The soil with *M. anisopliae* contained $1.2x10^6$ spores per gram dry soil in July and $0.5x10^6$ spores per gram dry soil at the end of the experiment (March 1992). Isolate H211 gave the best result and was even better than the chemical control.

The experiment in the climate rooms (III) show that temperature affects the efficacy of the nematode isolates. It also appears that the four isolates of *H. megidis* have a different efficacy at 12°C and 15°C. At 12°C isolate H211 performed best. Isolate HD appeared to be inferior to H211 and HSH at 15°C.

Table 2 - Percentage reduction of *O. sulcatus* larvae compared with "untreated" (untr.), achieved by carbofuran (carb.), *M. anisopliae* and six isolates of *Heterorhabditis* sp. under different conditions.

		Percentage reduction								
experiment	O.s.#	untr.	carb.	H211	HSH	HD	HF85	HI	HAu	BIO1020
container outdoors (I)	2.2	0a*	95de	96de	85c	-	99e	70bc	63b	85cd
open ground (II)	5.1	0a	40ab	73c	17a	-	-	60bc	-	52b
climate room (III)										
9°C	4.5	0a	-	29a	14a	4a	-	-	-	-
12°C	2.9	0a	-	57b	28ab	11ab	-	-	-	-
15°C	2.6	0a	-	90b	93b	29a	-	-	-	-

* *Figures in the same row followed by the same letter are not statistically significantly different, with a 95% confidence limit (square root transformation).*

\# *average number of O. sulcatus larvae in the untreated plants.*

Figure 1 shows the average daily temperature of the soil in pots (experiment I) and in the open ground (experiment II) from the first inoculation until three weeks after the second inoculation with nematodes. From this point until the end of the experiments temperature never exceeded 6°C. Except for one day, from the day of the first inoculation until 16 October the temperature varied between 11 and 14°C. After the latter date the temperature never exceeded 11°C and varied between 4 and 11°C. In the period between the first and second inoculations the soil temperature in the open ground was higher than in the pots.

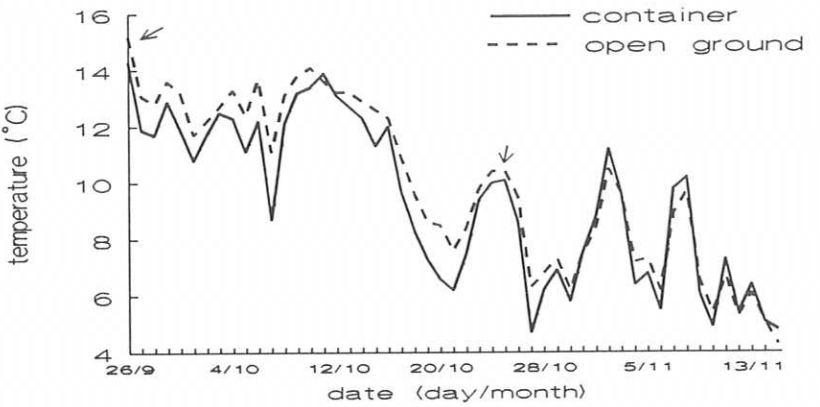

Figure 1 - Daily average soil temperature in containers and in the open ground in 1991 after inoculation with insect parasitic nematodes. Arrows indicate the days when nematodes were applied.

DISCUSSION

All the biological agents we tested successfully controlled the larvae of the black vine weevil in the container experiment. The nematode isolates H211 and HF85 and also the fungus *M. anisopliae* were as effective as the chemical treatment with carbofuran. The nematode isolates differ in efficacy. *H. bacteriophora* isolates (HI and HAu) were generally inferior to the *H. megidis* isolates (H211, HF85 and HSH).

In the open ground two isolates of nematodes (H211 and HI) and the fungus *M. anisopliae* gave good control, even outdoing carbofuran, which gave no statistically significant control. The open ground experiment was done in the same period as the container experiment. The same batches of nematode isolates and weevil eggs were used in both experiments. It is therefore remarkable to see that all agents were less effective against the larvae in the open ground than in the containers. Soil temperature cannot be the only reason for this difference in efficacy, as the results in figure 1 show; antagonism in the soil is probably a contributory factor. The soil in pots has a relatively poor microbial life compared with the soil in the field.

As the results show, carbofuran and one nematode isolate (HSH) did not reduce the number of larvae in the open ground experiment. Although HSH was also less effective than H211 in the container experiment it is remarkable that in contrast with H211 and HI this isolate was ineffective in the open ground. Antagonism in the field soil might have had an extra impact on HSH. The results from the climate room experiment show that temperature influences the efficacy of HSH. At 15°C both isolates (H211 and HSH) were effective and temperature was not limiting for a good control. At 12°C, however, only isolate H211 was effective compared with "untreated". The temperature limits for the HSH isolate differ from those for H211. In the open ground it is possible that the soil temperature in 1991 in combination with antagonism were unfavourable for effective control. Each nematode isolate has its own efficacy curve determined by the substrate and the temperature.

The fungus *M. anisopliae* can infect all stages of the weevil. The presence of enough spores in the soil in one season makes it possible to control the population of the larvae in the period that the weevil starts laying eggs until September. The spores are only moderately influenced by antagonism in soil and the experimental results showed that the spore concentration in the soil was never limiting (according to Stenzel, Bayer A.G.) although the concentration decreased over the season. Temperatures below 15°C limit successful infection (Hartwig & Oehmig, 1992). Infection and final death of the larvae proceed much more slowly than with nematodes and therefore not all larvae have been killed by the fungus by September. Additional use of cold-tolerant isolates of nematodes in this period would be a good strategy to obtain complete control of the black vine weevil.

It is essential to know more about the temperature characteristics of the various isolates of nematodes in different substrates. This would enable better advice to be given to growers on when to apply the nematodes. Future research will concentrate on these questions.

REFERENCES

ANDERSCH, W., HARTWIG, J., REINECKE, P. & K. STENZEL, 1990 - Production of Mycelial Granules of the Entomopathogenic Fungus *Metarhizium anisopliae* for Biological Control of Soil Pests. *Proc. of the 5th Int. Coll. on Invertebrate Path. and Microbial Control., Adelaide. Soc. Invert. Path.*, 25.

DOLMANS, N.G.M., 1983 - Biological control of the Black Vine Weevil (*Otiorhynchus sulcatus*) with a nematode (*Heterorhabditis* sp.). *Med. Fac. Landbouww. Rijksuniv. Gent* **48(2)**, 417-420.

DORSCHNER, K.W., AGUDELO-SILVA, F. & C.R. BAIRD, 1989 - Use of *Heterorhabditid* and *Steinernematid* nematodes to control Black Vine Weevils in Hop. *Florida Entomologist* **72(3)**, 554-556.

GEORGIS, R. & P.O. POINAR, 1984 - Greenhouse control of the black vine weevil *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) by *Heterorhabditid* and *Steinernematid* nematodes. *Environ. Entomol.* **13**, 1138-1140

HARTWIG, J. & S. OEHMIG, 1992 - BIO1020 - Behavior in the soil, and important factors affecting its action. *Pflanzenschutz-Nachrichten Bayer* **45**/1992,1, 159-176.

KLINGLER, J., 1988 - Investigations on the parasitism of *Otiorhynchus salicicola* and *O. sulcatus* (Col.: Curculionidae) by *Heterorhabditis* sp. (Nematoda). *Entomophaga* **33**, 325-331.

SHANKS,JR, C.H. & F. AGUDELO-SILVA, 1990 - Field Pathogenicity and Persistence of *Heterorhabditid* and *Steinernematid* Nematodes (Nematoda) Infecting Black Vine Weevil Larvae (Coleoptera: Curculionidae) in Cranberry Bogs. *J. Econ. Entomol.* **83(1)**, 107-110.

STOREY, G.K., MCCOY, C.W., STENZEL, K. & W. ANDERSCH, 1990 - Conidiation Kinetics of the Mycelial Granules of *Metarhizium anisopliae* (BIO1020) and its Biological Activity against Different Soil Pests. *Proc. of 5th Int. Coll. on Invertebrate Path. and Microbial Control, Adelaide. Soc. Invertebrate Path.*, 320-325.

BIOLOGICAL CONTROL OF LEATHERJACKETS WITH *BACILLUS THURINGIENSIS*

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Key words: *Tipula paludosa, Tipula oleracea*, biological control, *Bacillus thuringiensis* subsp. *israelensis*

Summary

Leatherjackets, larvae of *Tipula* species (Diptera: Tipulidae), are a major pest of grassland in NW-Europe. Laboratory bioassays showed that the larvae are sensitive to the insect pathogenic bacterium *Bacillus thuringiensis* subsp. *israelensis* (Bti). Older larvae become less sensitive to Bti. Field trials showed that first instar larvae can be controlled effectively with dosages of 45 l/ha of Skeetal and Vectobac, available commercial Bti products. Both dosage and time of application are unfavourable for large scale practical use, with a possible exception for sports fields and lawns. Laboratory and greenhouse tests with bait formulations showed reasonable effects against third instar larvae with dosages of 16 l/ha of commercial Bti products. Field tests with these bait-formulations and other commercial Bti productswill have to show the possibilities for large scale practical use of Bti for the biological control of leatherjackets in pastures.

INTRODUCTION

Leatherjackets, larvae of *Tipula* species (Diptera: Tipulidae), are a major pest in grassland in NW-Europe. The larvae feed on grass foliage rather than roots as is often assumed (Ricou, 1967; Vlug, 1990) and often cause severe damage to pastures (Blackshaw, 1984; Vlug 1992). The damage consists of direct reduction of grass yield but perhaps more important is the removal of nitrogen-rich grasses. This creates open spots in the pasture which are subsequently colonized by low-quality grasses or weeds, forcing the farmer to resow his pasture (Lauenstein, 1986). When numbers exceed damage thresholds of 150 larvae/m^2 in autumn or 100 larvae/m^2 in spring, chemicals such as parathion and chlorpyrifos are used for control (Vlug, 1992).

In NW-Europe two species, *T. paludosa* Meigen and *T. oleracea* L. are of major economic importance. Together with *T. czizecki* de Jong they form a species complex that can interbreed but has sterile offspring (den Hollander, 1975). *T. paludosa* is univoltine and the most dominant species. *T. oleracea* is less common but bivoltine and much easier to rear (Wiegers et al., 1992). The latter species was therefore used in bioassays to determine the susceptibility to *Bacillus thuringiensis* subsp.

israelensis (Bti). The asumption that the susceptibility of *T. paludosa* larvae would not differ greatly given the close relatedness of the two species, was later confirmed.

Bti is pathogenic for dipteran larvae of the suborder of Nematocera and used for the control of the larvae disease vectors such as *Aedes, Anopheles* and *Simulium* larvae (Krieg, 1986). Other potential targets among the Nematocera are Sciaridae. As the family Tipulidae belongs to the suborder Nematocera it is not surprising that Bti showed activity against *Tipula* species. Krieg (1986) mentioned activity of Bti against *Tipula*, but without data or references. Langenbruch (unpubl. results) and Waalwijk et al. (1992) confirmed the biological activity of Bti against *Tipula* larvae. Chard et al. (1990) reported on the first laboratory and field trials. This paper presents data on the dose-mortality relationships between Bti and *T. oleracea* larval instars, data on field trials with commercial Bti-products and experiments with bait formulations. The potential for practical use of Bti for biological control of leatherjackets is discussed.

MATERIALS AND METHODS

Leatherjacket rearing. The rearing method (Wiegers et al., 1992) is largely based on methods by Laughlin (1958) and Carter (1975). Gravid *T. oleracea* females were collected in pastures in the neighbourhood of Wageningen, the Netherlands. Egg laying is enforced by decapitation. The eggs are transferred to Petri dishes with 1.5% water-agar where they are allowed to hatch. The temperature is kept at 20°C with 16h light per day. Eggs hatch 7-10 days after oviposition and the larvae are fed on lettuce leaves. One week after hatching the petri-dishes usually become too wet and the larvae are transferred to glass dishes (ϕ 20 cm) half filled with moist coarse sand.

Culturing *Bacillus thuringiensis* subsp. *israelensis* (Bti). Bti-strain IPS-82 was obtained from the Institute Pasteur in Paris. A starting culture was inoculated with a single colony from a Nutrient-Broth-Agar (Oxoid) plate and cultured overnight at 37°C on a shaker at 340 rpm/min. Peptonized milk broth was inoculated with 1 ml/100 ml from the starting culture in LB-medium (trypton (Oxoid) 10 g/l, yeast extract (Oxoid) 5 g/l, NaCl 10 g/l) at 30°C on a shaker at 340 rpm/min for 3-4 days until complete sporulation (Ibarra & Federici, 1986).

Bioassays on *Tipula* larvae. Compartments of 6-well tissue-culture plates (Greiner) were filled with a layer of 1.5% water-agar (Oxoid). In each well a lettuce leaf disc (ϕ 16 mm) was placed on top of the agar and 10 µl of a Bti IPS-82 suspension in PBS (Phosphate Buffered Saline, Oxoid) with 0.025% Citowett spreader-sticker (Shell) was equally distributed over the surface with a spatula. After drying the leaf-discs with air from a ventilator a single *Tipula* larva was put into each well. The tissue-culture plates were covered with a layer of tissue-paper, closed with a lid and placed at 20°C in complete darkness for 7 days. Larval mortality was checked daily. When larvae had consumed the whole leaf-disc a fresh one without treatment was provided. Series of five concentrations around the LC-50 value were used. Each concentration was tested 3 times against 30 larvae for each of the four larval instar. The dose-mortality lines (Fig. 1) were calculated with a probit-analysis programme (Polo-PC, LeOra Software, California, USA).

The field trial was carried out on a lawn with plots of 1 m^2, each surrounded by a plastic barrier to prevent leatherjackets from migrating between plots. Treatments consisted ofthe application of two commercial Bti-products (flowable formulations), Skeetal (Novo-Nordisk, 600 IU/mg) and Vectobac (Abbot 1200 IU/mg) in volumes of 5, 15 45 and 135 l/ha (1 ml equals 1 gram). Each treatment was carried out in 3 repetitions on seperate plots. Bti was applied with a propane-driven handsprayer at 4 bar pressure, nozzle 120, in a volume of 500 ml/m^2 with an addition of 25 ml/100 litre of Citowett spreader-sticker. To each plot 200 first instar laboratory-reared *T. oleracea* were added. The experiment started on September 28th. After 4 weeks the top 15 cm of the whole plot was placed in a container with salt water (1 kg/5 l water) (after Vlug & Paul, 1986). The larvae floating on the surface were collected after 30 min. with a sieve and counted.

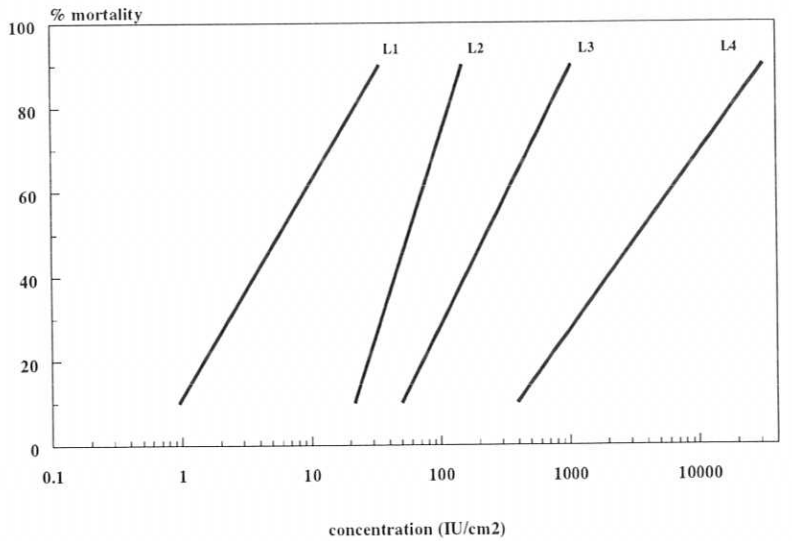

Fig 1. Dose-mortality relationship between Bti and T.oleracea larval instars (L1-L4).

Bait formulations. Grass-sods of 30x40x10 cm were dug out of a pasture with a natural population of third instar *Tipula* larvae and placed in trays in a greenhouse at a temperature of 15°C. Bti-pellets (100 IU/mg, Novo-Nordisk) were ground with a mortar. Wheat bran and wheat germs were spread evenly on a plate and sprayed with Bti. To each gram of wheat germs or bran 200 µl of Bti flowable concentrate (Novo-Nordisk, 900 IU/mg) was added. On each grass sod 1 gram of wheat bran or germs or 1.8 grams of ground Bti-pellet was evenly scattered by hand. This dosage corresponds with 16 litres of Bti product in 80 Kgs of wheat germs or bran or 144 kg of ground Bti-pellets per hectare, respectively. Each treatment was tested on five different sods. Control plots remained untreated. After two weeks the trays were checked for larvae with the salt water method.

RESULTS

The results of the bioassays with Bti against the four larval instars of *T. oleracea* show that with increasing age the larvae become less sensitive to Bti (Figure 1). Each subsequent larval instar roughly shows a 10-fold increase in LC-50.

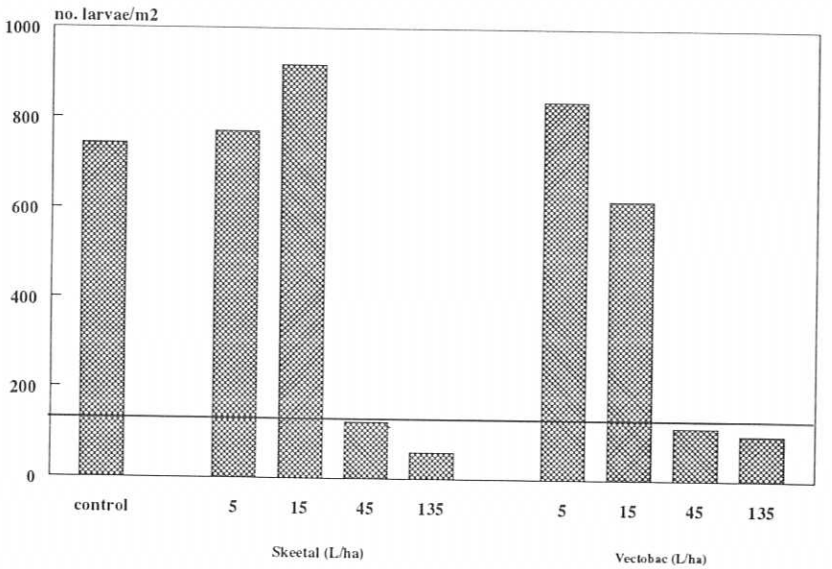

Fig 2. Field trials against first instar Tipula larvae with two Bti products.

Figure 2 shows the results of a field trial with two commercial Bti-products on a lawn, sprayed when first instar larvae were present. The results show clearly that a dosage of 45 litres per hectare of each the products gives sufficient control of first instar *Tipula* larvae to reduce the numbers below the damage threshold of 150 larvae/m^2 (horizontal line in Figure 2). Vectobac which should be twice as potent as Skeetal based on the amount of International Units per mg, does not give better results, the two products perform equally well. To each plot 200 larvae were added to ensure a sufficient population. The results show that the natural population was already very high with 600-700 larvae/m^2 and that the addition of larvae had not been necessary.

Table 1 shows the results of a greenhouse trial with three bait formulations against third instar *Tipula* larvae in natural grass-sods. All three bait formulations have an effect on the numbers of surviving larvae. The ground Bti-pellet gives the best results with 61 percent effect. The wheat germs mixed with Bti seem to do better than the wheat bran.

Table 1. Number of surviving larvae in greenhouse trials on grass-sods with natural populations of third instar Tipula larvae.

Treatment	Total no. larvae	average no. larvae	S.D.	% control
untreated control	106	21.2	9.0	0
wheat bran + Bti	89	17.8	6.9	16
wheat germs + Bti	53	10.6	3.4	50
ground Bti pellets	42	8.4	3.6	61

DISCUSSION

The results show that leatherjackets are sensitive to Bti and can be effectively controlled with commercially available Bti-products. The dosages required for effective control, in the order of 45 litres product per hectare, are however at this moment probably too high to allow wide-scale practical use from an economical point of view. The price of a litre Bti-product is difficult to assess and depends largely on price-market policy. Production costs are estimated at 1-10 dollars per litre product. This means a hectare treatment will cost at least several hundreds of dollars, which is probably more than farmers are willing to spend on the control of leatherjackets in pastures.

Another problem is the timing of the application. The results in figure 2 show the effect of Bti against first instar larvae. Other field trials (Smits, unpubl. results) showed that older larvae could not be effectively controlled by spraying volumes as high as 135 litres of Bti. First instar larvae are, however, extremely small and difficult to sample. Furthermore the oviposition by adult females occurs throughout the months of August and September depending on weather conditions. In addition numbers fluctuate strongly from year to year. Nowadays farmers are supposed to sample their pastures by taking soil samples analyzed with the salt-water method (Vlug & Paul, 1986) at the end of November or in early spring. By that time the larvae are big enough to be seen and counted in the muddy salt water. First instars are too small to be counted reliably with this method and secondly the timing of the sampling is difficult. Also high numbers of first instars in September do not necessarily mean high numbers and damage in the following spring. The only option would be preventive application of Bti against first instars, based on information of the numbers of adults flying, combined with knowledge on the general population levels, which follow a seven-year cycle (Lauenstein, 1986). This would require years of research and may not prove to be reliable.

It seems much more sensible to aim at late autumn and preferably early spring application against third instar leatherjackets. The current sampling methods can then be used by farmers. The problem is that against older larvae higher dosages of Bti are required. The solution can lie in the use of other formulations such as bait-formulations or more concentrated flowable formulations or wettable powders. The third instar leatherjacket larva needs to acquire a lethal Bti-dose within a rather short period of several hours because Bt in general has an anti-feeding effect (Krieg,

1986). Sublethal dosages will lead to cessation of feeding by the larvae which will recover after a couple of days and then resume feeding again (van Frankenhuizen, pers. comm.). The persistence of effective dosages of Bt on foliage lies in the order of only 4-5 days. The formulations used in our experiments were developed for control of mosquito larvae in rivers and not for use on grass. Specially developed formulations may considerably improve the performance of Bti. Our experiments with bait-formulations show that even with lower dosages per hectare good control of third instars can be achieved although this has to be confirmed in field tests.

In conlusion there is a good potential for the practical use of Bti to control leatherjackets in pastures, lawns and sports fields. A lot of work still has to be done on the formulation of Bti-products specifically developed for use on grass against leatherjackets and possibly bibionids as well. But with a market of several millions of hectares in NW-Europe and N-America and increasing pressure to abandon the use of chemical control agents it seems likely that these products will be developed in the near future.

REFERENCES

Blackshaw, R.P., 1984. The impact of low numbers of leatherjackets on grass yield. *Grass and Forage science* 39: 339-343.

Carter, J.B., 1975. Rearing *Tipula oleracea* (Diptera, Tipulidae) in laboratory cultures. *Pl. Path.* 24: 101-104.

Chard, J.M., R.G. McKinlay & J. Baty, 1990. Observations on the effects of *Bacillus thuringiensis* subsp. *israelensis* on crane fly larvae. *Aspects of Appl. Biol.* 24: 277-278.

Hollander, J. den, 1975. The phenology and habitat of the species of the subgenus *Tipula* Linnaeus in the Netherlands (Diptera, Tipulidae). *Tijdschr. Ent.* 118: 83-97.

Ibarra, J.E. & B.A. Federici. 1986. Isolation of a relatively nontoxic 65-kilodalton protein inclusion from the parasporal body of *Bacillus thuringiensis* subsp. *israelensis*. *J. Bacteriol.* 165: 527-533

Krieg, A., 1986. *Bacillus thuringiensis*, ein mikrobielles Insektizid. Paul Parey Publ., Berlin, 191 p.

Lauenstein, G., 1986. Tipuliden als Grünlandschädlinge. - Biologie und Bekämpfung. *Z. Ang. Zool.* 4/86: 385-431.

Laughlin, R., 1958. The rearing of crane flies (Tipulidae). *Ent. exp. & appl.* 1:241-245.

Ricou, G., 1967. L'alimentation des larves des tipules (*Tipula paludosa* Meig.). *Ann. Nutr. Aliment.* 21: 199-215.

Vlug, H.J. & H. Paul, 1986. Sampling leatherjackets. *Med. Fac. Landbouww. Rijksuniv. Gent* 51: 939-942.

Vlug, H.J., 1990. Feeding behaviour of Tipulid larvae on grass. *Meded. Fac. landbouww. Rijksuniv. Gent* 55: 545-547.

Vlug, H.J., 1992. Emelt graast weiland af. *Boerderij* 78 (10): p 48

Waalwijk, C., A. Dullemans, G. Wiegers and P. Smits. Toxicity of *Bacillus thuringiensis* variety *israelensis* against tipulid larvae. *J. appl. Ent.* 114: 415-420.

Wiegers, G.L., A.M. Dullemans & J. Wijbenga, 1992. The rearing of *Tipula oleracea* L. (Dipt., Tipulidae). *J. Appl. Ent.* 114: 410-414.

VIRUS-VECTOR INTERACTIONS DURING THE TRANSMISSION OF TOSPOVIRUSES BY THRIPS

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Key words: Tospovirus, tomato spotted wilt virus, *Frankliniella occidentalis*, transmission, latent period, propagative virus

Summary

Using a novel assay system, based on the formation of local lesion on *Petunia X hybrida*, we showed that a large fraction of the viruliferous thrips could transmit two tospovirus isolates in the second larval stage and that the LP_{50} decreased with increasing temperatures. In addition we showed that the virus multiplies in thrips.

INTRODUCTION

Tospoviruses, which are exclusively transmitted by thrips (Thysanoptera; Thripidae) in a persistent manner, cause worldwide serious diseases of various crops. Eight thrips species have been reported as vectors, of which *Frankliniella occidentalis* (Pergande), is apparently the most important one. Virus acquired by larvae renders the thrips infectious, while transmission of the virus is mainly ascribed to adults (Sakimura, 1962). The mechanism by which the virus is transmitted is not understood. Parameters expressing the transmission quantitatively do not exist.

Studies on tospovirus transmission are hampered by the lack of readily applicable techniques. Therefore, an assay was developed using leaf discs of the local lesion host *Petunia X hybrida* which allowed an efficient measurement of quantitative aspects of the transmission and handling of the thrips. This assay was employed to quantify transmission efficiency and latent period of two tospoviruses transmitted by *F. occidentalis* at 3 different temperatures.

To gain further insight in the mechanism of tospovirus transmission the fate of virus in the vector was investigated. Conclusive evidence for multiplication of tospoviruses in thrips can be obtained by detection of the non-structural proteins, NSs or NSm, which do not occur in the virus particles but whose presence depend on the replication of the viral genome (Kormelink *et al.*, 1991; 1992), and by demonstrating an increase of the nucleocapsid (N) protein. Therefore, newly hatched first instar larvae, which acquired virus for only 2 hours on infected plants, were sampled at several moments after this acquisition for their virus content by ELISA using antisera to the N protein and the nonstructural protein (NSs). In addition, evidence is obtained by immunolabeling that the salivary glands are a major site of virus multiplication in thrips.

MATERIAL AND METHODS

Thrips. Virus-free *F. occidentalis* were reared on bean pods (*Phaseolus vulgaris* L. cv Prelude) at 27 ±0.5°C and 16 h photoperiod (L/D:16/8). The colony was started with adults collected from a greenhouse infestation in the Netherlands.

Virus isolates. The BR-01 isolate of tomato spotted wilt virus (TSWV) and the NL-07 isolate of *Impatiens* necrotic spot virus (INSV) were used. TSWV is the type species of the newly established tospovirus genus within the family of the Bunyaviridae. INSV, a serological distinct TSWV-like virus, mainly found in ornamental plant species, was isolated from *Impatiens* (Avila *et al.* 1992).

Acquisition feeding. *Impatiens* sp. plants, used as virus source, were infected 2 to 3 weeks after sowing by single viruliferous adults of *F. occidentalis* carrying either the TSWV isolate BR-01 or the INSV isolate NL-07. The plants were grown in a greenhouse at approx. 22°C (L/D: 16/8 h) for symptom development. Systemically infected leaves were used for acquisition feeding. First instar larvae, 0-4 h old, were confined to the surface of infected leaves using cages as described by Tashiro (1967). Larvae, caged on virus free *Impatiens*, were used as controls.

Latent period experiments. The thrips were given an acquisition access period (AAP) of 24 h at 3 different temperatures: 20 (±0.5), 24 (±0.5) and 27 (±0.5)°C. After the AAP, each larva was transferred every 24 h to a fresh leaf discs (Ø 13 mm) of *Petunia X hybrida* cv. Blue Magic (Allen and Matteoni, 1991) in a 1.5 ml Eppendorf tube at the same temperature as at which they acquired the virus. After each inoculation access period (IAP) of 24 h, the leaf discs were incubated at 27°C in 24 well plates, while floating on water for the development of local lesions. The latent period (LP) was defined as the time interval from the start of the AAP to the end of that IAP in which the first transmission occurred. The LP_{50} was estimated by log-probit analysis of the time-series of cumulative percentages of thrips transmitting the virus for the first time (Sylvester 1965). The LP_{50} and its 95% fiducial limit (FL) were calculated following Finney (1962).

Study of virus multiplication in thrips. The thrips were given an AAP of 2 h at 27 (±0.5)°C. After the AAP the larvae were transferred to healthy leaves of *Datura stramonium* in leaf cages. For ELISA a subgroup of thrips was collected and frozen at -70 °C directly after the AAP (2h). To perform the ELISA, samples of approx. 30 larvae were frozen at 6h intervals up to 48 h and at 60 and 72 h after the onset of AAP.

Selection of viruliferous thrips. To select viruliferous adults for light microscopic studies, adults grown from larvae exposed to infected plants for 24 h, were tested on leaf discs of *Petunia* X *hybrida* cv. Blue Magic at 24 °C as described previously.

Enzyme-linked immunosorbent assay (ELISA) with enzyme amplification. Individual thrips were analyzed both by cocktail-ELISA (N protein) and by antigen coated plates (ACP)-ELISA (NSs protein). Individual larvae were placed in Eppendorf tubes and triturated with a micropestle in 100 µl of sample buffer. The suspension was divided in 2 portions in order to analyze one thrips with two antisera. The ELISA was performed as described by Van den Heuvel and Peters (1989) with some alterations. For cocktail ELISA, wells were coated with 150 µl of 0,5 µg/ml polyclonal antiserum raised against the N protein in coating buffer. After blocking, one portion of the thrips sample was transferred to the wells and mixed with 50 µl of 1 µg/ml anti-N-conjugate in sample buffer. For ACP-ELISA, wells were coated with the second portion of the

thrips sample mixed with an equal volume of coating buffer (2X) and incubated overnight at 4 °C. Coating was subsequently followed by blocking, incubation of the wells with 100 μl of 0.4 μg/ml of polyclonal antiserum raised against NSs protein in sample buffer (2 h at 37 °C), and by incubation with 0.3 μg/ml goat anti-rabbit IgG alkaline phosphatase conjugate in sample buffer (2 h at 37 °C). Last steps of both ELISA's involved the enzyme amplification reaction.

Light microscopy. Thrips were immersed in 2% (w/v) paraformaldehyde and 3% (w/v) glutaraldehyde in 0.1 M $Na_2HPO_4.2H_2O$, 9.7 mM citric acid, pH 7.2, 1.5 mM $CaCl_2$. A small piece of the anterior part of the head and posterior part of the abdomen were cut off to allow penetration of the fixative into the tissues. The fixative was infiltrated under vacuum (70 mBar) for 2 h at room temperature (RT) and for another 16 h at 4°C under atmospheric pressure. The thrips were washed 6 times 10 min in demineralized water and dehydrated in a series of ethanol at low temperature; in 30% at 0°C for 30 min, in 50% at 0°C for 30 min and subsequently at -20°C for 30 min, in 70, 80, 90% and 2 times 100% at -20°C for 1 h. Thrips were then infiltrated with a 1:1 mixture of ethanol and LR Gold (London Resin Company) for 16 h and pure LR Gold for 48 h (refreshing the medium after 24 h) at -20°C. The specimens were transferred to BEEM capsules and polymerized with ultraviolet light (wavelength 360 nm) for 24 h at -25°C and 48 h at RT. Sections (1 μm thick) were individually mounted in drops of distilled water with glycerin albumen (Gurr) (1:200 v/v) onto glass slides pretreated with dimethyldichloro-silane. The slides were dried on a hot plate at 60°C. The sections were stained for 3 min in 1% toluidine blue in distilled water, washed in water, dried and mounted in Eukitt. Immunogold labelling and silver enhancement was done according to van Lent and Verduin (1987). Sections were examined with phase contrast or with epi-illumination in a Leitz Laborlux S light microscope equipped with a polarization filterblock (epipolarization microscopy). N and NSs protein antisera were used in a concentration of 1 μg/ml.

RESULTS AND DISCUSSION

Since thrips are not readily recovered form plants, a local lesion assay has been developed in which the thrips were allowed to feed on leaf discs from *Petunia X hybrida* cv. Blue Magic plants. This species reacts with the formation of small black or brown local lesions within two or three days after inoculation (Allen & Matteoni, 1991). Each viruliferous thrips evoked none to an uncountable number of local lesions on a disc within an IAP of 24 h. The results presented here show that an efficient assay system has been developed to test the infectivity of thrips larvae and adults. The INSV and TSWV lesions develop within 2 to 3 days after infection, and can readily be distinguished from feeding scars caused by the thrips. Since the larvae are very active and tend to move to the ground before pupation, assaying the larvae on a leaf disc is a more elegant method that the use of test plants.

Since the majority of the larvae transmitted virus, a LP_{50} could be determined before pupation. The LP_{50} and the 95% fiducial limits at the 3 temperatures applied, were estimated by log-probit analysis (Table 1). The LP_{50} decreased with increasing temperature. The LP_{50} values for the 3 temperature treatments significantly differed ($P < 0.05$). The temperature effects on the latent period have either to be explained by a higher replication rate or an increased movement of virus through the vector.

The identical LP_{50} values for INSV and TSWV show that the infectivity of the

Table 1. *Median latent period of larvae of* F. occidentalis *and efficiency of transmission of INSV isolate NL-07 and TSWV isolate BR-01 at 20, 24 and 27°C after an AAP of 24 h on systemically infected* Impatiens *leaves. Larvae were transferred every 24 h to fresh* Petunia *leaf discs.*

Temperature (°C)	LP_{50}[a]		Transmitting larvae (%)[c]		Transmitting adults (%)[d]	
	INSV	TSWV	INSV	TSWV	INSV	TSWV
20	157 (150-163)[b]	171 (164-177)	80.0	52.8	92.5	55.1
24	103 (96-108)	109 (103-116)	70.0	39.7	85.0	44.9
27	82 (78- 87)	84 (77- 89)	63.3	32.9	81.6	43.0

[a] Median latent period (LP_{50}); the time interval at which 50% of the larvae of *F. occidentalis* completed their latency period (LP). The LP is defined is the period of time between the start of the AAP and the end of the IAP in which the first virus transmission took place

[b] Between brackets; 95 % fiducial limits of the LP_{50}

[c] Thrips that already started to transmit virus in the second larval stage.

[d] The total number of thrips that already started virus transmission in the second larval stage and the number of thrips that started to transmit virus for the first time in the adult stage.

thrips develops at the same rate. The LP_{50}'s of INSV and TSWV found are considerable shorter and show a much smaller variation than reported for TSWV in the past (Sakimura, 1962). A few adults that did not transmit as larvae, transmitted virus shortly after emergence. The LP of these thrips is probably finished at the end of the second larval stage or during prepupal or pupal stage.

At the 3 temperatures applied, the majority of the viruliferous thrips already transmitted virus during the second larval stage (Table 1). When larvae reached the prepupal stage, they stopped feeding. As a result of this behaviour no virus was transmitted during this and the pupal stage. The adults which emerged resumed feeding. Thrips that already transmitted virus as larvae, transmitted the virus also in the adult stage. The results obtained showed that INSV is transmitted more efficiently than TSWV. The efficiencies by which the tospoviruses are transmitted, are also influenced by host susceptibility. Because high transmission rates were found in our experiments we conclude that both the local lesion host (*Petunia*) and systemic host (*Impatiens*) are very suitable plant species for performing transmission studies.

Multiplication of viruses in their vectors can be demonstrated by quantitatively assaying the increase of virus specific antigens in their vectors. Monitoring of the N protein would give a measure for the accumulation of virus particles in thrips, while monitoring of the NSs protein would provide evidence for expression and replication of the viral genome. Cocktail-ELISA with polyclonal antibodies against the N protein showed that the titer of this protein dropped within the first hours after acquisition and increased thereafter to levels above the amount ingested and achieving a plateau before becoming a prepupae (Fig. 1). Lower values for the N protein were consistently observed around 36 h after acquisition; a phenomenon which coincided with moulting of the larvae from the first instar to the second one. While the increase of N protein in the thrips larvae already indicated that TSWV multiplies in its vector, further evidence for this was obtained by demonstrating the *de novo* synthesis of the NSs protein. The

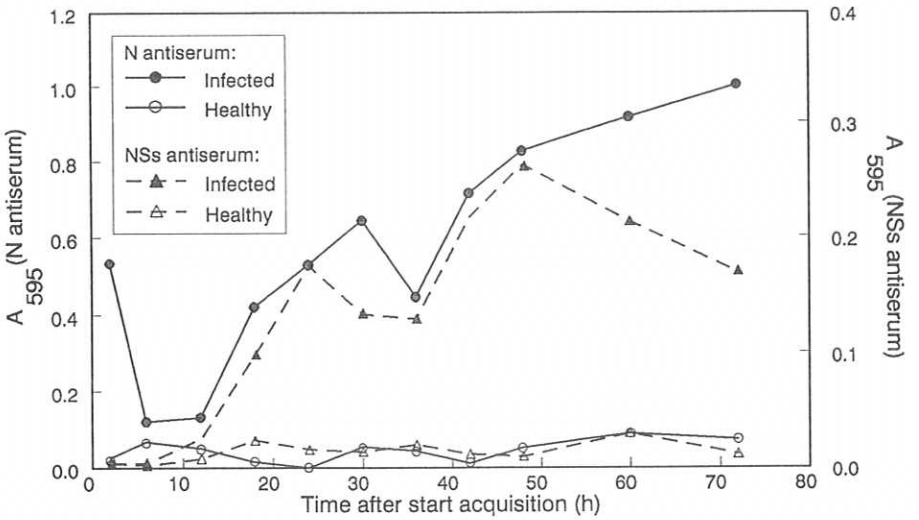

Fig. 1. Production of nucleocapsid (N) protein (continuous lines) and the non-structural (NSs) protein (dotted lines) of TSWV in larvae of F. occidentalis *after a 2 h acquisition access period. The mean ELISA values obtained at the various intervals from 30 thrips which were singly tested, are given.*

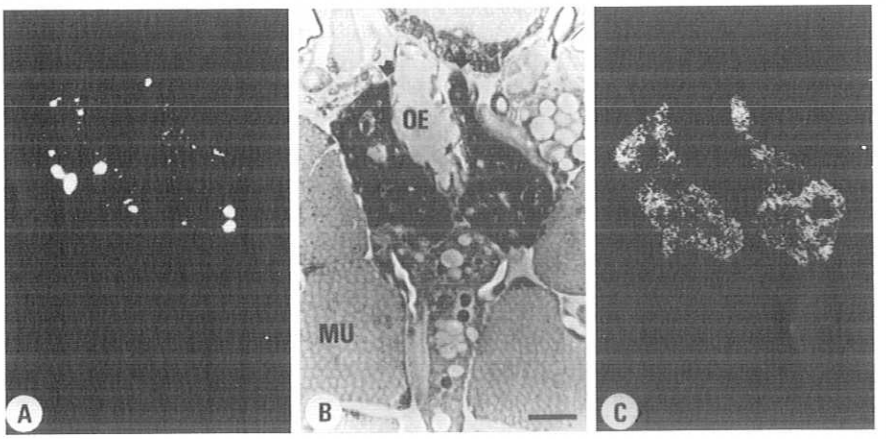

Fig. 2. Serial sections of the salivary gland (arrows) of an adult thrips which acquired the virus in the first larval instar. (A) Epipolarization microscopy of the section after immunogold/silver staining with N antiserum. (B) Phase contrast image of the same area. (C) Epipolarization of the section following immunogold/silver staining with NSs antiserum. MU=muscle; OE=oesophagus. Bar represents 10 μm.

amount of this protein detected in thrips after acquisition was virtually zero but started to increase simultaneously, though at a dissimilar rate, with the N protein (Fig. 1).

In situ localization of virus and viral proteins in thrips was done with adults as they contained readily detectable amounts of N and NSs protein (results not shown). Abundant amounts of the N and NSs proteins occurred in the salivary gland tissue (Fig. 2). Epipolarization studies showed the presence of the N protein in confined areas of the gland tissue (Fig. 2A), while the NSs protein appears equally spread through the cells (Fig. 2C).

Electron microscopic studies revealed the presence of many electron-dense aggregates (viroplasms) embedded in the cytoplasm of infected gland cells which were specifically gold labelled using N antiserum and which showed structural similarities to the viroplasms in infected plant cells (Kitajima *et al.*, 1992). Numerous virus particles were observed in the salivary ducts, aligned to the duct membrane. The saliva vesicles accumulating in the salivary gland cells contained only a limited number of virus particles. Another, prominent location of both N and NSs proteins were the muscle cells associated with the midgut epithelium (results not shown).

The *in situ* localization of the N and NSs proteins in salivary glands and in midgut muscle cells presents further evidence for multiplication of TSWV in thrips and provides information on the sites of active replication. The increase of the N and NSs proteins firmly demonstrates replication of TSWV in its vector. As expected, the accumulation of these proteins coincides closely with the development of infectivity of the larvae.

REFERENCES

ALLEN, W.R. & J.A. MATTEONI, 1991 - Petunia as an indicator plant for use by growers to monitor for thrips carrying the tomato spotted wilt virus in greenhouses. *Plant Disease* **75,** 78-82.

DE ÁVILA, A.C., P. DE HAAN, E.W. KITAJIMA, R. KORMELINK, R. DE O. RESENDE, R.W. GOLDBACH and D. PETERS, 1992 - Characterization of a distinct isolate of tomato spotted wilt virus (TSWV) from *Impatiens* sp. in the Netherlands. *Journal of Phytopathology* **134,** 133-151.

FINNEY, D.J., 1962 - Probit analysis, a statistical treatment of the sigmoid response curve. Cambridge University Press, 318 pp.

KITAJIMA, E.W., A.C. DE ÁVILA, R. DE O. RESENDE, R.W. GOLDBACH & D. PETERS, 1992 - Comparative cytological and immunogold labelling studies on different isolates of tomato spotted wilt virus. *Journal of Submicroscopy and Cytological Pathology* **24**, 1-14.

KORMELINK, R., E.W. KITAJIMA, P. DE HAAN, D. ZUIDEMA, D. PETERS & R. GOLDBACH, 1991 - The non-structural protein (NSs) encoded by the ambisense S RNA segment of tomato spotted wilt virus is associated with fibrous structures in infected plant cells. *Virology* **181**, 459-468.

KORMELINK, R., P. DE HAAN, C. MEURS, D. PETERS & R. GOLDBACH, 1992 - The nucleotide sequence of the M RNA segment of tomato spotted wilt virus, a bunyavirus with two ambisense RNA segments. *Journal of General Virology* **73**, 2795-2804

SAKIMURA, K., 1962 - The present status of thrips-borne viruses. In Biological transmission of disease agents, pp 33-40. Ed. Maramorosch, K. Acad. Press, New York.

SYLVESTER, E.S., 1965 - The latent period of pea-enation mosaic virus in the pea aphid, *Acyrthosiphon pisum* (Harris) - an approach to its estimation. *Virology* **25,** 62-67.

TASHIRO, H., 1967 - Selfwatering acrylic cages for confining insects and mites on detached leaves. *Journal of Economical Entomology* **60,** 354-356.

VAN DEN HEUVEL, J.F.J.M., T.M. BOERMA & D. PETERS, 1991 - Transmission of potato leafroll virus from plants and artificial diets by *Myzus persicae*. *Phytopathology* **81,** 150-154.

VAN LENT, J.W.M. & B.J.M. VERDUIN, 1987 - Detection of viral antigen in semi-thin sections of plant tissue by immunogold-silver staining and light microscopy. *Netherlands Journal of Plant Pathology* **93**, 261-272.

MICROSPORIDIOSIS IN MASS REARINGS OF PREDATORY MITES: DEVELOPMENT OF A DETECTION METHOD

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Key words: microsporidia, Pleistophoridae, monoclonal antibodies, *Amblyseius* sp., Acari, mass rearing

Summary

Until now, three microsporidian species have been found in mass rearings of the predatory mites *Amblyseius cucumeris* and *A. barkeri*. Against spores of one species (Pleistophoridae), which infects both predatory mites and their prey mites in the rearings, two hybrid cell lines producing monoclonal antibodies were developed. These antibodies will be applied in an ELISA to screen mass rearings for microsporidiosis, and in histological studies for detection and identification of microsporidia.

INTRODUCTION

Microsporidia (Phylum: Microspora) are obligatory intracellular protozoans, which can be found in all major groups of the animal kingdom (*e.g.*, insects, fish, man) (Bulla & Cheng, 1976). They are very common pathogens of invertebrates, but until a few years ago only one microsporidium infecting mites was described: *Nosema steinhausi* in *Tyrophagus noxius* (Weiser, 1956). At the end of the previous decade however, microsporidiosis was established as the cause of a decrease in productivity of mass rearings of *Amblyseius cucumeris* and *A. barkeri* (W. Ravensberg & M. Dissevelt, Koppert Biological Systems, pers. comm., 1988; Ramakers *et al.*, 1989). These predatory mites are used in greenhouses for the control of thrips (*Frankliniella occidentalis* and *Thrips tabaci*), which can be serious pests in various vegetables (*e.g.*, Ramakers *et al.*, 1982). A strong reduction in number and quality of predatory mites may endanger the biological control of thrips, and when pesticides have to be used instead, it may upset integrated pest management programs in greenhouses.

Several mass rearings of *A. cucumeris* and *A. barkeri* have been examined for microsporidiosis. So far, three microsporidian species have been found in rearings used for commercial or experimental purposes. One species was detected in predatory mites as well as in the stored product mites *Acarus siro* and *Tyrophagus putrescentiae*, which are the prey in mass rearings (Beerling & van der Geest, 1991). This microsporidium with small, oblong spores (spore measures: 1.8µm x 0.9µm) belongs to the Pleistophoridae family (A.M. Huger, Institut für Schädlingsbekämpfung, Darmstadt, BRD, pers.

comm., 1991). A second species which turns up regularly in mass rearings, has small, more oval spores (1.4µm x 0.8µm) and is, so far, exclusively found in prey mites (E.B., unpubl. res., 1992). Only occasionally a third species with large spores (2.6µm x 1.3µm) is detected in mass rearings. This species is found in prey mites only and is probably *Nosema steinhausi* (A.M. Huger, pers. comm., 1990).

Screening rearings for microsporidiosis is laborious and demands skill, because prey- and predatory mites have to be checked individually by light microscopy to detect and identify microsporidian spores. Therefore, we tried to find an alternative method that makes screening for microsporidia easier, faster and more reliable.

Immunoassays seem to be very efficient in screening for insect pathogens (Oien & Ragsdale, 1992) and are likely to satisfy these demands. In few studies, serological techniques are employed for detection or identification of microsporidian spores. Niederkorn *et al.* (1980) and Sato *et al.* (1981) make use of indirect fluorescent antibody techniques (IFAT) and Irby *et al.* (1986) apply immunoblot assays to test immunological relationships between spores of different microsporidian species. Greenstone (1983) was the first to use an enzyme-linked immunosorbent assay (ELISA) in connection with microsporidiosis, and since then others have applied this technique for spore detection (Hollister & Canning, 1987; Whitlock & Brown, 1991) or identification (Kawarabata & Hayasaka, 1987; Oien & Ragsdale, 1992). Of all serological assays, ELISA is probably the most efficient technique for screening mass rearings because of its ability to process large quantities in a simple, rapid, sensitive and quantitative manner (Engvall & Perlmann, 1972).

In most immunoassays, polyclonal antibodies are used. In only a few serological studies monoclonal antibodies - against spores of *Nosema bombycis* - are applied (Mike *et al.*, 1988; Ke *et al.*, 1990; Iwano & Ishihara, 1991). Monoclonal antibodies are more specific than polyclonal antibodies. Furthermore, a hybridoma cell line will produce one kind of antibody with a constant quality and for indefinite time, whereas polyclonal antibodies are only available in limited quantities and changing qualities. For these reasons, we opted for developing monoclonal antibodies against microsporidian spores from mites. These antibodies will be used in an ELISA to screen predatory mite mass rearings for microsporidiosis. In addition, they also will be of value in histological studies for detecting spores and identifying species in slide- or smear preparations. For the purpose of identification, it is important to know if the monoclonal antibodies show any cross-reactivity with spores of other microsporidia from mites. More generally, for classification of new microsporidian species, data on cross-reactivity with spores of many species are helpful.

For practical reasons, this study concentrates on the two most prevalent microsporidia from mass rearings of predatory mites: the one with small, oval spores and the other one with small, oblong spores (Pleistophoridae). The third species (*Nosema steinhausi*) was only rarely found in mass rearings and therefore perforce left out of consideration.

MATERIALS AND METHODS

Mass rearing

The predatory mites *Amblyseius cucumeris* and *A. barkeri* are reared on stored product mites (*Acarus siro* or *Tyrophagus putrescentiae*), in containers with wheat bran (Ramakers & van Lieburg, 1982). The prey mites live on yeast and on fungi that grow on wheat bran (water content 20%$^{v/w}$). The relative humidity in the containers is approximately 90% and the temperature ranges from 22° to 24°C. Samples used for isolation of spores were kindly provided by Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands).

Isolation of microsporidian spores

Spores of two microsporidian species (the small oblong microsporidium from the Pleistophoridae family and the small oval species) were isolated from samples of the mass rearing. Mites were sieved from the culture medium (wheat bran and yeast) using tap water and four sieves (500µm - 250µm - 90µm - 63µm), and crushed in a glass tissue grinder. The suspension was filtered through 1 cm cotton wool in a syringe to remove large particles (cuticulae). Spores were purified by centrifugation at 16000g for 20 minutes through a 40% silica colloid (Ludox) density gradient (Undeen & Alger, 1971). Subsequently, the fractions with spores were washed with water to remove the Ludox (1700g; 20 min.) and spores were counted in a haemocytometer (Bürker-Türk) and stored in phosphate-buffered saline (PBS) at -20°C.

For cross-reactivity tests *Nosema bombi*, *N. bombycis* and *Vairimorpha necatrix* were available. Spores of *N. bombi* were isolated from mid- and hindguts of highly infected *Bombus terrestris* workers. Guts were ground in a glass tissue grinder and spores were purified from this suspension according to the method described above. Spores of *Vairimorpha necatrix* and *N. bombycis* were obtained through Dr. C.R. Vossbrinck (University of Illinois, Urbana, IL, USA) and Dr. T. Kawarabata (Kyushu University, Higashi-ku, Japan) respectively.

Production of antibodies

Two BALB/c mice were intraperitoneally injected with 200 µl antigen (10^6 spores of both species in PBS), emulsified in Freund's incomplete adjuvant (1:1). This was repeated after 30 days and again after 14 days. Three days prior to spleen isolation for cell fusion, the mice received intraperitoneally a final booster injection of spores in PBS without Freund's adjuvant. Two weeks after each injection, blood samples were taken to determine the antiserum titre. Isolated spleen cells of both mice were fused with Sp2/0-Ag-14 myeloma cells according to Schots *et al.* (1992). Hybridoma cells producing spore-specific antibodies were cloned at least twice (Köhler & Milstein, 1975).

Immunofluorescence microscopy

An indirect fluorescent antibody technique (IFAT) was used to determine antisera titres of blood samples and to screen tissue culture supernatants for the presence of spore-specific antibody production. Spores suspended in water were brought on 24 wells glass slides ($3x10^4$ spores/well) and air-dried. Sera (serially diluted in PBS) and supernatant were examined for antibodies against both spores using FITC(=Fluoresceine)-conjugated goat anti-mouse antibody. Slides were screened with an immunofluorescence microscope.

Cross-reactivity of monoclonal antibodies with spores of *Nosema bombi*, *N. bombycis* and *Vairimorpha necatrix*, was tested with the same IFAT.

RESULTS

Polyclonal antisera from the first bleeding reacted moderately (dilution up to 1:500), and antisera from subsequent bleedings and fusion blood reacted strongly (dilution up to 1:10,000) in an IFAT to spores of both microsporidian species. Six fusions revealed two clones producing monoclonal antibodies (MJ1 and MJ2) directed against the oblong spores (of the Pleistophoridae family). They did not show any reaction with the small, oval spores of the other microsporidium from mass rearings. Only a weak cross-reactivity was observed between MJ1 or MJ2 and spores of *Nosema bombi*, and no reaction with spores of *N. bombycis* or *Vairimorpha necatrix* (table I).

Table I: Reactivity of monoclonal antibodies (MAb) MJ1 and MJ2 (developed against oblong spores (Pleistophoridae sp.) from mites) with spores of several microsporidia. Symbols represent respectively: ++, strong; +, moderate; ±, weak; –, no reaction in IFAT.

MAb:	oblong spores from mites	oval spores from mites	spores of *N. bombi*	spores of *N. bombycis*	spores of *V. necatrix*
MJ1	++	–	±	–	–
MJ2	+	–	±	–	–

DISCUSSION

The first cell lines producing monoclonal antibodies against spores of a microsporidium (*Nosema bombycis*) were obtained by Mike *et al.* (1988). Shortly hereafter, Ke *et al.* (1990) succeeded in producing two different monoclonal antibodies against spores of *N. bombycis*. In the present study monoclonal antibodies against a second microsporidium species were realized. These antibodies MJ1 and MJ2 were found to be specific for spores of one of the microsporidia (of the Pleistophoridae family) from mass rearings. This microsporidian species was detected not only in prey mites, but also in the predatory mites of mass rearings (Beerling & van der Geest, 1991). With the development of MJ1 and MJ2, we have a tool for detecting and identifying this harmful microsporidium.

Because it is difficult to purify large numbers of microsporidian spores, we used an IFAT for determining antisera titres of blood samples and for screening hybridoma cells on spore-specific antibody production. An advantage of this technique is that far less spores are required compared to the amounts needed for an ELISA. In addition, an IFAT can be applied in taxonomical and histological studies on microsporidia in mites.

For screening mass rearings of predatory mites on microsporidiosis, an ELISA is adequate. Therefore, it is important that MJ1 and MJ2 react only with microsporidian spores, and not with mite cells or other material from mass rearings (*e.g.*, yeast, fungi). Preliminary results show no cross-reactivity of MJ1 or MJ2 with ground material from uninfected mass rearings.

For identification and classification purposes it is essential to assess the specificity of the monoclonal antibodies, since antibodies may be directed against conservative as well as specific epitopes (spore surface antigens). For this reason we tested MJ1 and MJ2 for cross-reactivity with spores of other microsporidian species. Hitherto, reactions with spores of only four species have been examined. A weak cross-reactivity with spores of *Nosema bombi* was found, but unfortunately monoclonal antibodies against these spores are not available for reciprocal tests. As yet, we can only speculate about the existence of any antigenic similarities between spores of *N. bombi* and the oblong spores from mass rearings. Before any statements about the specificity of MJ1 and MJ2 can be made, cross-reactivity with many other microsporidian spores should also be tested.

Currently, hybridoma cell lines that produce monoclonal antibodies against the second microsporidian species (with small, oval spores) are developed. If these monoclonal antibodies-to-be are combined with the new antibodies against the oblong spores (and maybe with monoclonal antibodies against *Nosema steinhausi*), the result will be a powerful and sensitive screening method for microsporidia in mass rearings.

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REFERENCES

BEERLING, E.A.M. & L.P.S. VAN DER GEEST, 1991. Microsporidiosis in mass-rearings of the predatory mites *Amblyseius cucumeris* and *A. barkeri* (Acarina: Phytoseiidae). *Proc Exper. &. Appl. Entomol., NEV Amsterdam* **2**: 157-162.

BULLA, L.A. & T.C. CHENG (eds.), 1976. *Comparative Pathobiology. I: Biology of the Microsporidia.* 371pp. Plenum Press, New York.

ENGVALL E., & P. PERLMANN, 1972. Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. *J. Immunol.* **109** (1): 129-135.

GREENSTONE, M.H., 1983. An enzyme-linked immunosorbent assay for the *Amblyospora* sp. of *Culex salinarius* (Microspora: Amblyosporidae). *J. Invertebr. Pathol.* **41**: 250-255.

HOLLISTER, W.S. & E.U. CANNING, 1987.An enzyme-linked immunosorbent assay (ELISA) for detection of antibodies to *Encephalitozoon cuniculi* and its use in determination of infections in man. *Parasitology* **94**: 209-219.

IRBY, W.S., Y.S. HUANG, C.Y. KAWANISHI & W.M. BROOKS, 1986. Immunoblot analysis of exospore polypeptides from some entomophilic microsporidia. *J. Protozool.* **33** (1): 14-20.

IWANO, H. & R. ISHIHARA, 1991. Dimorphic development of *Nosema bombycis* spores in gut epithelium of larvae of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Japan.* **60** (4): 249-256.

KAWARABATA, T. & S. HAYASAKA, 1987. An enzyme-linked immunosorbent assay to detect alkali-soluble spore surface antigens of strains of *Nosema bombycis* (Microsporida: Nosematidae). *J. Invertebr. Pathol.* **50**: 118-123.

KE, Z., W. XIE, X. WANG, Q. LONG & Z. PU, 1990. A monoclonal antibody to *Nosema bombycis* and its use for identification of microsporidian spores. *J. Invertebr. Pathol.* **56**: 395-400.

KÖHLER, G. & C. MILSTEIN, 1975. Continuous cultures of fused cells secreting antibody of predifined specificity. *Nature* **256**: 495-497.

MIKE, A., M. OHWAKI & T. FUKADA, 1988. Preparation of monoclonal antibodies to the spores of *Nosema bombycis*, M11 and M12. *J. Sericult. Sci. Japan.* **57**: 189-195. [in Japanese with English summary]

NIEDERKORN, J.Y., J.A. SHADDUCK & E. WEIDNER, 1980. Antigenic cross-reactivity among different microsporidan spores as determined by immunofluorescence. *J. Parasitol.* **66** (4): 675-677.

OIEN, C.T. & D.W. RAGSDALE, 1992. A species-specific Enzyme-Linked Immunosorbent Assay for *Nosema furnacalis* (Microspora: Nosematidae). *J. Invertebr. Pathol.* **60**: 84-88.

RAMAKERS, P.M.J., M. DISSEVELT & K. PEETERS, 1989. Large scale introductions of phytoseiid predators to control thrips on cucumber. *Med. Fac. Landbouww. Rijksuniv. Gent* **54** (3a): 923-929.

RAMAKERS, P.M.J. & M.J. VAN LIEBURG, 1982. Start of commercial production and introduction of *Amblyseius mckenzie* sch. & pr. (Acarina: Phytoseiidae) for the control of *Thrips tabaci* lind. (Thysanoptera: Thripidae) in glasshouses. *Med. Fac. Landbouww. Rijksuniv. Gent* **47** (2): 541-545.

SATO, R., M. KOBAYASHI, H. WATANABE & T. FUJIWARA, 1981. Serological discrimination of several kinds of microsporidian spores isolated from the silkworm, *Bombyx mori*, by an indirect fluorescent antibody technique. *J. Sericult. Sci. Japan.* **50** (3): 180-184.

SCHOTS, A., R. POMP & W.B. VAN MUISWINKEL, 1992. Production of Monoclonal Antibodies. In: *Techniques in Fish Immunology.* (Stolen J.S., T.C. Fletcher, D.P. Anderson, S.L. Kaattari & A.F. Rowley, eds.): 1-18. SOS Publications, Fair Haven, NJ.

UNDEEN, A.H. & N.E. ALGER, 1971. A density gradient method for fractionating microsporidian spores. *J. Invertebr. Pathol.* **18**: 419-420.

WEISER, J. 1956. *Nosema steinhausi* n. sp., nová mikrosporidie z roztoče *Tyrophagus noxius* (Acarina, Tyroglyphidae). *Ceskoslovenská parasitol.* **3**: 187-193. [in Czech with German summary]

WHITLOCK, V.H. & S.T. BROWN, 1991. First record for *Nosema locustae* in the Brown Locust *Locustana pardalina* in South Africa, and the yield of spores in laboratory bioassays. *J. Invertebr. Pathol.* **58**: 164-167.

NOSEMA INFECTION IN HONEYBEES (*Apis mellifera* L.) AND BUMBLEBEES (*Bombus terrestris* L.)

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Key words: *Apis mellifera*, *Bombus terrestris*, *Nosema apis*, *Nosema bombi*, Protozoa, Microsporidia

Summary

Nosema spores were isolated from honeybees (*Apis mellifera* L.) and bumblebees (*Bombus terrestris* L.). Spores have been fed to the broodstages and adults of *Apis mellifera* and *Bombus terrestris*. Infections have been checked 10-12 days after feeding the spores. *Nosema apis* L. did not infect bumblebees and *Nosema bombi* did not infect honeybees. *Nosema bombi* is characterized by the facts that:

- only the larval stage of *Bombus terrestris* L. can be infected.
- spores of *Nosema bombi* in bumblebees develop not only in the midgut but also in the Malpighian tubules.
- spores are oval shaped and measure 5 µm long.

It is possible to rear *Bombus terrestris* colonies free of Nosema, if young queens are selected from clean colonies and hygiene is taken care of during the rearing process.

INTRODUCTION

For several years now bumblebees (*Bombus terrestris* L.) are being used for the pollination of glasshouse tomatoes. Colonies of *Bombus terrestris* are reared in climate rooms by several companies on a commercial scale. These colonies are used for pollination in glasshouses throughout Europe (Ruijter A. de, 1992). Large amounts of pollen are used for feeding the colonies. This pollen is honeybee collected pollen. Honeybees are furthermore used in stimulating the bumblebee queen in starting a broodnest (Heemert, C. van et al., 1990). As a result of the close contact between honeybees and bumblebees, diseases and parasites could interact. Although it is claimed that infection of *Bombus terrestris* L. with *Nosema apis* is not possible (Uspenskii, 1949), cross-infection of *Bombus fervidus* with *Nosema apis* was reported (Showers et al, 1967). The bumblebee colonies used in this experiment were free flying colonies and the spores found could result from a natural infection by *Nosema bombi*. Furthermore for the tests, honeybees were used which had been treated with fumagillin. This does not garantee that the bees were free of *Nosema apis* at the beginning of the reinfection tests.

Nosema is a disease of the adult honeybee. The disease is caused by a spore-forming Protozoa of the order Microsporidia: *Nosema apis* Zander (1909). This parasite develops in the tissue of the midgut. Nosema occurs all over the world. In the Netherlands and Belgium Nosema is often reported. The damage to bee colonies is considerable. Spores are oval shaped and measure 6 µm long. Spores are orally ingested by licking contaminated combs and by drinking contaminated water and honey. The spores are quickly passed from

the proventriculus to the midgut (Kellner, N. & Jacobs, F.J. 1978). Inside the midgut a polar filament is extruded and through this filament the parasite infects a cell of the midgut. Through several stages inside the cell (Fries, 1988) new spores develop. Dead cells containing spores end up in the midgut and the spores leave the beebody with the faeces. Little is known about Nosema occurring in bumblebees. *Nosema bombi* is described as a species by Fantham & Porter (1914). The following description of *N. bombi* is taken from a paper by Kudo (1924): "Habitat: Alimentary canal and Malpighian tubules of *Bombus agrorum, B. hortorum, B. latreillelus, B. lapidarius, B. sylvarum, B. terrestris*". Not all authors make a distinction between *Nosema apis* and *N. bombi* (Bulla, L.A. Jr. & Cheng, T.C., 1977). Experience with bumblebee colonies at the Ambrosiushoeve learned that spores of *Nosema bombi* can be found not only in males, females and queens, but also in pupae and freshly emerged adults. In infected colonies the viability of the bumblebees decreases, the life span is shortened and less young queens are produced, in comparison with uninfected colonies (Eijnde, J. van den, A. de Ruijter & J. Barten, unpublished).
Spores of *Nosema bombi* in *Bombus terrestris* can also be found in the Malpighian tubules, whereas *Nosema apis* can only be found in the tissue of the midgut. Spores of *Nosema bombi* are oval shaped and measure 5 µm long.

MATERIAL AND METHODS

Experiments took place in the autumn of 1992.
Nosema apis. Honeybee midguts were squashed on object slides. In case spores were detected, squashed midguts were washed from the slides with water and homogenised. The spore suspension was diluted with sucrose solution (72%) and fed to honeybees. The bees were kept in an incubator at 25°C. After 24 hours the suspension is replaced by a sucrose solution (50%). After one week new spores have developed. Midguts are squashed in a mortar. The obtained mass is diluted with water and the number of spores per ml is counted using a Bürker count chamber .
Nosema bombi. The infected bumblebees originate from outside. The reared colonies remain in the climate room and bumblebees taken from these colonies showed no infection of nosema. The alimentary canal between proventriculus and hindgut of infected *Bombus terrestris* is squashed, diluted with water and sprinkled onto larvae in the broodnest of bumblebee colonies (*Bombus terrestris*). Colonies are kept at 29°C in a climate room continuously. After 3 weeks sufficient infected bumblebees are available to obtain a spore suspension of *Nosema bombi*, the same way as described above for *Nosema apis*.
Apis mellifera L.
Adult honeybees.
A broodcomb containing emerging bees is removed from a colony and placed in an incubator at 34.5° C. After 4 hours the newly emerged bees are collected. These bees are fed with a sucrose solution (50%) and bee bread. Bee bread was kept at 60° C for two hours in order to kill any Nosema spores present. The adult honeybees are infected by offering them a suspension of spores in sucrose solution.
Spores are distributed among the bees by means of trophallaxis. The honeybees are kept in small wooden cages with glass walls in a climate room at 29°C.
After a minimum time period of one week the midguts were checked for spores. The bees of the negative control group are fed sucrose solution only.
Honeybee larvae.
A number of full grown, uncapped larvae are marked on an overhead projector sheet which is fixed on the frame by means of two thumb tacks. These larvae are infected by dripping

one drop of suspension onto each larva. The larvae of the negative control group received a drop of water instead. After removing the sheet (leaving the thumb tacks onto the frame), the comb is returned into the colony. After 12 days, just before the young bees emerge, the pupae or young bees are removed from the cells and the midguts are checked for spores.

Bombus terrestris L.

Adult bumblebees.

Adult bumblebees are collected from colonies in the climate room. The bees are divided into three groups. In the first group of bumblebees the midguts are directly checked for Nosema spores.

The other bumblebees are let to starve for a period of 4 hours. In order to infect the bumblebees a spore suspension is offered to the individual bees. This is necessary because bumblebees do not show any trophallaxis. The bees in the negative control group are fed with sucrose solution only. The bumblebees are put back together again and kept in small cages in a climate room at 29°C. After at least 10 days the midguts are checked for spores.

Bumblebee larvae.

A group of larvae in a bumblebee colony is sprinkled with a spore suspension. The group is located as good as possible on a drawn map of the broodnest. After 12 days the clump of pupae is removed. It is likely that more pupae are removed than there have been infected. The pupae or young bees are removed from the cells and the midguts are checked for spores.

RESULTS AND DISCUSSION

No spores were found in the midguts (n=78) of the adult bumblebees that were checked directly after their collection from the colonies. So it became clear that the test colonies were free of Nosema.

-*Apis mellifera* L. infected with *Nosema apis* Z.

Nosema apis exclusively develops in adult honeybees.

-*Bombus terrestris* L. infected with *Nosema apis* Z.

Spores of *Nosema apis* are not capable to infect adult bumblebees or larvae.

-*Bombus terrestris* L. infected with *Nosema bombi.*

Spores fed to adult bumblebees do not result in an infection.

Larvae infected with *Nosema bombi* develop an infection which is shown in the pupae and emerged bees.

-*Apis mellifera* L. infected with *Nosema bombi.*

No infection have been developed in adult honeybees.

Larvae contaminated with Nosema bombi do not develop an infection which is shown in the pupae and emerging bees.

Table 1: % nosema infected

	honeybees *Apis mellifera*		bumblebees *Bombus terrestris*	
	adult	pupae	adult	pupae
Nosema apis	100 % (n=37)	0 % (n=39)	0 % (n=38)	0 % (n=48)
Nosema bombi	0 % (n=17)	0 % (n=86)	0 % (n=34)	73 % (n=113)
negative control	0 % (n=56)	0 % (n=102)	0 % (n=83)	

Nosema apis L. did not infect bumblebees (*Bombus terrestris* L.) and *Nosema bombi* did not infect honeybees (*Apis mellifera* L.) .

Nosema apis and *Nosema bombi* are indeed different species.
Nosema bombi is characterized by the facts that:
- only the larval stage of *Bombus terrestris* L. can be infected.
- spores of *Nosema bombi* in bumblebees develop not only in the midgut but also in the Malpighian tubules.
- spores are oval shaped and measure 5 µm long.

It is possible to rear *Bombus terrestris* colonies free of Nosema, if young queens are selected from clean colonies and hygiene is taken care of during the rearing process.

REFERENCES

BULLA, L.A. Jr. & CHENG, T.C., 1977 - Comparative Pathobiology Volume 2 Systematics of the Microsporidia *Plenum Press . New York and London*

FRIES, I., 1988 - Contribution to the study of Nosema disease (Nosema apis Z.) in honeybee (Apis mellifera L.) colonies. *Dissertation*, Report 166, Sveriges Lantbruksuniversitet, Uppsala.

HEEMERT, C. VAN, A DE RUIJTER, J. VAN DEN EIJNDE, J VAN DER STEEN, 1990 - Year-round production of bumblebee colonies for crop pollination. *Bee World* **71**, 54-56.

KELLNER, N. & F.J. JACOBS, 1978 - In hoeveel tijd bereiken de sporen van Nosema apis Zander de ventriculus van de honingbij (Apis mellifera L.)? *Vlaams Diergeneeskundig Tijdschrift* **47**, 252-259.

KUDO, R., 1924 - A biologic and taxonomic study of the microsporidia. Illinois Biol. Monogr. **9**, 9-60, 90-101, 103-5.

RUIJTER, A. DE, 1992 - Recent Developments in Insect Pollination of Horticultural Crops. Bumblebeequest **2** (2), 1-4.

SHOWERS, R.E., A. JONES & F.E. MOELLER, 1967 - Cross-Inoculation of the Bumble bee *bombus fervidus* with the Microsporidian *Nosema apis* from the Honey Bee. Journal of Economic Entomology **60**(3), 774-777.

USPENSKII, V. D., 1949 - Bumblebees and nosema disease of honeybees. Works of the XXVII Sess. of the Vet. Sect. All-Union Lenin Agr. Acad. 168: 93-97. Biol. Abstr. **27**, 7914 (1953).

PRIMARY INFECTION IN *SPODOPTERA EXIGUA* LARVAE USING A RECOMBINANT *AUTOGRAPHA CALIFORNICA* NUCLEAR POLYHEDROSIS VIRUS

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In Lepidopteran larvae the primary infection of baculoviruses takes place in the midgut. Here, the polyhedra dissolve and release the infectious virus particles. These particles pass through the peritrophic membrane, fuse with the columnar cell microvilli membrane and the nucleocapsids are transported to the nucleus (1). In order to study the early events of baculovirus infection an *Autographa californica* nuclear polyhedrosis virus recombinant (AcMO16B) was constructed. This recombinant contained the bacterial ß-galactosidase gene under control of the Drosophila heat-shock promoter. This construct allows histochemical detection of ß-galactosidase as early as 4 h post infection (p.i.) in cultured *Spodoptera frugiperda* cells. This implies that viral infection can be detected this way already a few hours before the start of viral DNA replication.
Second-instar *Spodoptera exigua* larvae were fed with polyhedra of AcMO16B and the midgut was examined histochemically to locate the viral infection. Infection was observed as early as 6 h p.i. It showed that only columnar cells were infected at this time. The infection was predominantly located in the anterior part of the midgut. At 12 h p.i. a secondary infection was established in midgut regenerative cells and also in some cells of the haemocoel. At this time no tissue response, like the rejection of infected columnar cells or proliferation of regenerative cells, was observed. At 24 h p.i. a few infected columnar cells were rejected from the epithelium, confirming former investigations (2). Apparently this tissue response occurred to late to abort infection.

1) Granados, R.R., and Lawler, K.A., 1981. Virology, **108**, 297-308.

2) Flipsen, J.T.M., van Lent, J.W.W., Goldbach, R.W., and Vlak, M.J., 1992 J. Invertebr. Pathol., in press.

SEX ATTRACTANTS AND THE TAXONOMIC STATUS OF *DICHRORAMPHA GUINÉE*

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Key words: Dichrorampha, electroantennograms, pheromone traps, speciation, sympatry, Torticidae.

Summary

Sex attractants for *Dichrorampha* Guenée males (Lepidoptera, Tortricidae) were tested in field trapping experiments and with electroantennogram recordings. E9,11-12:Ac (E9, 11 - dodecadienyl acetate) appeared to be an attractant to the *Dichrorampha* males studied in the field. This compound also induces high responses in electroantennogram recordings of the tested species, suggesting that E9,11-12:Ac is an important component of the sex pheromones of *Dichrorampha* species.

According to the literature as well as our findings, E9,11-12:Ac uniquely attracts Olethreutinae belonging to *Dichrorampha*. It can probably be regarded as an apomorphy for *Dichrorampha*, supporting the monophyly of the genus. This conclusion justifies the suggestion that sex pheromone variation has played a role in the speciation events that led to the separation of *Dichrorampha* and its sister group.

INTRODUCTION

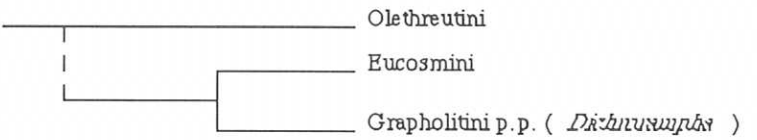
Biological, i.e. non-morphological, characters of *Dichrorampha* Guenée species were studied to check their correlation with the current classification of the genus. This study is part of a research programme in which biological data are incorporated into phylogenetic studies in an attempt to formulate effect analyses for processes of speciation.

The *Dichrorampha* species that we studied are sympatric, and the moths have overlapping flight periods. Host plant associations and variation in pheromone components have been shown by various authors (see Tauber & Tauber, 1989 and Cardé, 1987 and the references therein) to play a key role in the reproductive isolation of sympatric insect populations, reason why both aspects are studied.

CLASSIFICATION AND BIONOMICS

Dichrorampha classified in the lepidopteran family Tortricidae, subfamily Olethreutinae, tribe Grapholitini, is Holarctic in its distribution and comprises about 115 described species, 17 of which are Nearctic.

Due to insufficient insight in the higher classification of the Tortricidae, *Dichrorampha*'s sister group is uncertain. Horak and Brown (1991) suggest that the genus originated from a more generalized Olethreutini, a tribe that they consider as a paraphyletic group from which Eucosmini and some Grapholitini, the tribe to which *Dichrorampha* belongs, have evolved.



Dichrorampha species have a restricted number of host plants: their larvae feed only in the roots, the rootstocks and/or stems of a few plant species belonging to the family Compositae (Ulenberg, 1992).

Table 1. Dichrorampha *species occurring in The Netherlands with their host plants.* The host plant associations are based on rearing experiments and on literature (see for the references Ulenberg, 1992).

Dichrorampha species	host plant
D. sedatana Busck	*Chrysanthemum vulgare*
D. plumbana (Scopoli)	*Achillea millefolium*, *Chrysanthemum leucanthemum*
D. aeratana (Pierce & Metcalfe)	*Chrysanthemum leucanthemum*, *Achillea millefolium*
D. plumbagana (Treitschke)	*Achillea millefolium*
D. obscuratana (Wolff)	*Achillea millefolium*, *Ac. ptarmica*, *Chrysanthemum vulgare*
D. agilana (Tengström)	*Chrysanthemum vulgare*, *Achillea millefolium*
D. petiverella (L.)	*Achillea millefolium*, *Ac. ptarmica*, *Chrysanthemum vulgare*, *C. corymbosum*
D. alpinana (Treitschke)	*Chrysanthemum leucanthemum*, *C. vulgare*, *C. coronarium*, *Achillea millefolium*
D. flavidorsana Knaggs	*Chrysanthemum vulgare*
D. simpliciana (Haworth)	*Artemisia vulgaris*, *Artemisia* sp., *Senecio jacobea*
D. consortana Stephens	*Chrysanthemum leucanthemum*
D. sylvicolana Heinemann	*Achillea ptarmica*
D. acuminatana (Lienig & Zeller)	*Chrysanthemum vulgare*, *C. leucanthemum*, *C. segetum*
D. gueneeana Obraztsov	*Achillea millefolium*, *Chrysanthemum vulgare*, *C. leucanthemum*

This study has been performed with the 14 species occurring in The Netherlands. Those species with their host plants are listed in Table 1. As already stated above, these *Dichrorampha* species are sympatric and the moths have overlapping flight periods (Table 2). Nothing is known, however, about the periods of sexual activity of the different species. Each of the plant species, except *Artemisia vulgaris,* harbour more than one *Dichrorampha* species. Moreover, in rearing experiments different *Dichrorampha* species appeared to develop simultaneously in individual plants.

Table 2. Flight periods of Dichrorampha *species in The Netherlands.* Data are based on collecting dates of the specimens of this genus in museum and private collections in The Netherlands (Kuchlein, in press).

Dichrorampha species	flight period				
	May	June	July	August	September
D. sedatana	++++++++++++++++				
D. plumbana	++++++++++++++++++++++++++				
D. aeratana	++++++++++++++++				
D. plumbagana	++++++++++++++++				
D. obscuratana	++++++++++++++++++++++++++++++				
D. agilana	++++++++++++++++++++++				
D. petiverella	++++++++++++++++				
D. alpinana				+++++++	
D. flavidorsana	++++++++++++++++++++++++++++++				
D. simpliciana	++				
D. consortana	++++++++++++++++				
D. sylvicolana		++++++++++++++++++++			
D. acuminatana	++				
D. gueneeana	++++++++++++++++++++++++++++++				

MATERIAL AND METHODS

Sex attractants for *Dichrorampha* males as mentioned by Mayer & McLaughlin (1991) were tested in field trapping experiments, using UNI-traps, and with electroantennogram recordings.

Field experiments. E9- and Z9-12:Ac, E9- and Z9-12:OH and an isomeric blend of E/Z9,11-12:Ac were tested separately and in 1:1 binary mixtures. The E/Z9,11-12:Ac was in a mixture of 80 : 20 E/Z. All components had an isomeric purity of more than 98%. The UNI-traps were placed at 20-meter spacings, 1 meter above ground level in habitats of *Dichrorampha* during the summer of 1992, i.e.:

a) Wrakelberg, Limburg, habitat with *Leucanthemum vulgare* and *Achillea millefolium*, 6-22 June, and 9 August - 27 September,
b) Uffelte, Drente, habitat with *Tanacetum vulgare* and *Achillea millefolium*, 1 July - 30 September,
c) Teuge, Gelderland, habitat with *Tanacetum vulgare*, *Achillea millefolium* and *Artemisia vulgaris*, 1 July - 30 September,
d) Hilversum, Noord-Holland, habitat with *Artemisia vulgaris*, 25 August - 30 September (one trap with E/Z9,11-12:Ac).

The traps were checked and cleaned once a week. The attractant dispensers in the traps were refreshed on 1 August.

Electroantennograms. Electroantennograms (EAGs) were recorded from male antennae of 5 *Dichrorampha* species, i.e.

- *aeratana* (8 specimens collected at the Wrakelberg)
- *petiverella* (1 specimen reared from larva ex *Achillea millefolium* from Teuge)
- *flavidorsana* (2 specimens reared from larvae ex *Tanacetum vulgare* from Uffelte)
- *simpliciana* (1 specimen reared from larvae ex *Tanacetum vulgare* from Maarland, Limburg)
- *gueneeana* (2 specimens reared from larvae ex *Tanacetum vulgare* from Teuge, and ex *Achillea millefolium* from Echt, Limburg)

EAGs were recorded on stimulation with 34 potential sex attractants using standard

EAG recording techniques. The responses were expressed as a percentage of the response to a reference stimulus, Z-2-hexen-1-ol (fig. 1).
The tested compounds were E5- and Z5-12:Ac, E5- and Z5-12:OH, E6- and Z6-12:Ac, E6- and Z6-12:OH, E7- and Z7-12:Ac, E7- and Z7-12:OH, E8- and Z8-12:Ac, E8- and Z8-12:OH, E9- and Z9-12:Ac, E9- and Z9-12:OH, E10- and Z10-12:Ac, E10- and Z10-12:OH, 11-12:Ac, 11-12:OH, E/Z9,11-12:Ac (in a mixture of 80 : 20 E/Z), E9,11- and Z9,11-12:Ac, Z9-14:Ac, Z9,E12-14:Ac, E11- and Z11-14:Ac and Z11-14:OH. All chemicals used for these recordings have a purity of > 99%.

RESULTS

Table 3. Number of Dichrorampha *males of various species attracted by different chemical compounds in UNI-traps in habitats of* Dichrorampha *in The Netherlands.*
The dates at which the specimens were found in the traps are between brackets. U.= Uffelte, Drente; W.= Wrakelberg, Limburg; T.= Teuge, Gelderland; H.= Hilversum, Noord-Holland.

	E9-12:OH E9-12:Ac	E9-12:OH Z9-12:OH	E9-12:OH EZ9,11-12:Ac	E9-12:Ac 100%	E9-12:Ac Z9-12:Ac	E9-12:Ac EZ9,11-12:Ac	Z9-12:Ac EZ9,11-12:Ac	Z9-12:OH EZ9,11-12:Ac	EZ9,11-12:Ac 100%
sedatana 6		1 W. (22 VI)				2 U. (10 VII)			
"						3 T. (10-18 VII)			
plumbana 4	1 U. (3 VII)			1 U. (3 VII)		1 T. (10 VII)			1 U. (3 VII)
aeratana 6			3 W. (16-22 VI)						3 W. (16 VI)
plumbagana 0									
obscuratana 0									
agilana 1			1 U. (18 VII)						
petiverella 2					1 T. (3 VII)			1 T. (24 VII)	
alpinana 5						2 W. (16 VI)		2 W. (8-16 VI)	1 W. (8 VI)
flavidorsana 2						1 U. (6 VIII)			1 U. (6 VIII)
simplicana 8									2 T. (18 VII+6 VIII)
"									6 H. (26 VIII-10 IX)
consortana 0									
sylvicolana 1							1 T. (3 VII)		
acuminatana 33			13 W. (16 VIII-27 IX)			2 W. (23+30 VIII)	1 W. (6 IX)	10 W. (30 VIII-27 IX)	5 W. (16-30 VIII)
"						1 U. (1 VIII)			
"						1 T. (6 VIII)			
guenceana 126			1 W. (16 VI)					1 W. (22 VI)	
"			10 U. (10 VII-1 VIII)	1U. (10 VII)		10 U. (3 VII-1 VIII)	2 U. (10 VII)	7 U. (10 VII-1 VIII)	2 U. (10-24 VII)
"			7 T. (3-24 VII)		1 T. (18 VII)	66 T. (3 VII-1 VIII)	1 T. (10 VII)	16 T. (3 VII-1 VIII)	1 T. (24 VII)
TOTAL 194	**1 (1 sp.)**	**1 (1 sp.)**	**35 (4 spp.)**	**2 (2 sp.)**	**2 (2 sp.)**	**89 (6 spp.)**	**5 (3 spp.)**	**37 (4 spp.)**	**22 (7 spp.)**

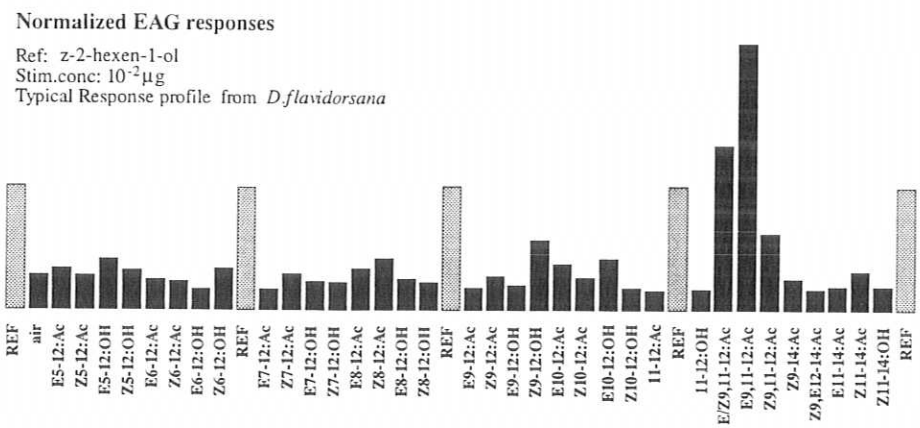


Figure 1.

Field experiments. Most *Dichrorampha* males were attracted by traps baited with E/Z9,11-12:Ac and with a mixture of E/Z9,11-12:Ac with either E9- or Z9-12:OH, or E9- or Z9-12:Ac (Table 3). No males were attracted by traps baited with E9-12:OH 100%, E9-12:OH + Z9-12:Ac, E9-12:Ac + Z9-12:OH, Z9-12:Ac 100%, Z9-12:Ac + Z9-12:OH and Z9-12:OH 100%. Of all species *D. gueneeana* and *D. acuminatana* were captured in the largest numbers, 126 and 33 individuals respectively. *D. plumbagana*, *D. obscuratana* and *D. consortana* were never observed in the traps. The other species came into the traps in low numbers, ranging from 1 to 8 individuals.

Electroantennograms. The EAG response spectra revealed similar profiles for all species tested. In figure 1 the response profile from *D. flavidorsana* is depicted, being representative for the other tested species. The maximum response was consistently evoked by E9,11-12:Ac alone followed by the isomeric mixture of E9,11-12:Ac and Z9,11-12:Ac.

DISCUSSION

The E/Z9,11-12:Ac mixture appeared to be an attractant to males of 11 out of the 14 studied *Dichrorampha* species in the field. The same compounds both induce high responses in electroantennogram recordings of the tested species, with E9,11-12:Ac evoking the highest response suggesting this to be an important component of the sex pheromones of *Dichrorampha* species. The high numbers of *D. gueneeana* males attracted by the mixture of E/Z9,11-12:Ac and E9-12:Ac may indicate that this mixture resembles the sex pheromone of *D. gueneeana*. The reason for the absence of *D. plumbagana* and *D. obscuratana* males in the traps is unclear. Both species are known to occur in Uffelte. *D. consortana* probably does not occur in the tested fields, which is in agreement with its absence in the traps.

The species attracted in the traps belong to different clades in the cladogram based on morphological characters of all the described *Dichrorampha* species, suggesting those species cover most of the variation within the genus (a paper on the phylogeny of *Dichrorampha* is in preparation). We may, therefore, consider the findings in this study to be representative for *Dichrorampha*.

According to the literature as well as our findings, E9,11-12:Ac (and Z9,11-12:Ac) is unknown to attract other Olethreutinae than *Dichrorampha* species.

Like most Olethreutinae *Dichrorampha* uses 12-carbon components in its sex pheromones. The 12-carbon chain structures in the Olethreutinae are thought to be derived from the 14-carbon chain structures predominantly found in the subfamily Tortricinae (Roelofs & Brown, 1982). Large numbers of Olethreutinae species use a Δ8, a Δ8,10 or a Δ9 system.

In the Olethreutini -from which *Dichrorampha* is thought to be evolved (see above)- a whole range of structures is found, i.e. 12- and some 14-carbon chains with a Δ8, Δ8,10 or Δ9 system. In the Eucosmini, in which tribe probably the sister group of *Dichrorampha* can be found, the Δ8 and the Δ9 systems are used. Most of the Grapholitini, the tribe in which *Dichrorampha* is classified, use the Δ8 system, exceptionally the Δ9 system. However, none of the Olethreutinae (so also none of the Olethreutini, the Eucosmini and the Grapholitini!) except *Dichrorampha* uses the Δ9,11 system.

Roelofs & Brown (1988) suggest that the Δ8, Δ8,10 and Δ9 desaturase systems in the pheromone biosynthesis routes in Lepidoptera have evolved prior to the Δ11 system. This hypothesised evolutionary route leads to the suggestion that E9,11-12:Ac is a derived character, probably derived from a E9-12:Ac chain. E9,11-12:Ac consequently can be regarded as an apomorphy for *Dichrorampha*, thus supporting the monophyly of the

genus. This conclusion justifies the suggestion that the sex pheromone variation has played a role in the speciation event leading to the separation of *Dichrorampha* and its sister group. The answer to the question of whether pheromone variation did initiate this speciation event is the ultimate aim of this research. In this context *Dichrorampha* appears to be very interesting, being sympatric with different congenerics in different parts of its distribution area, sharing host plants and flight periods with close relatives. Those conditions are ideal to investigate phenomena like radiation and character displacement.

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REFERENCES

Cardé, R.T., 1987. The role of pheromones in reproductive isolation and speciation of insects. In: *Evolutionary Genetics of Invertebrate Behavior* (M.D. Huettel, ed.): 303-317. Plenum Press, New York.

Horak, M. & R. L. Brown, 1991. Taxonomy and Phylogeny. In: *Tortricid Pests, Their Biology, Natural Enemies and Control* (L.P.S. van der Geest & H.H. Evenhuis, eds.): 23-48. Elsevier Science Publishers, Amsterdam.

Kuchlein, J.H., in press. *De Kleine Vlinders. Handboek voor de Faunistiek van de Nederlandse Microlepidoptera*, 715 pp. Pudoc, Wageningen.

Mayer, M.S. & J.R. McLaughlin, 1991. *Handbook of Insect Pheromones and Sex Attractants*, 1083 pp. CRC Press.

Roelofs, W.L. & R.L. Brown, 1982. Pheromones and evolutionary relationships of Tortricidae. -*Annual Review of Ecology and Systematics* **13**, 395-422.

Roelofs, W.L. & W.A. Wolf, 1988. Pheromone biosynthesis in Lepidoptera. -*Journal of Chemical Ecology* **14**(11), 2019-2031.

Tauber, C.A. & M.J. Tauber, 1989. Sympatric speciation in insects: perception and perspective. In: *Speciation and its Consequences* (D. Otte & J.A. Endler, eds): 307-344. Sunderland/Ma: Sinauer.

Ulenberg, S.A., 1992. The significance of host plant association in the speciation of Dichrorampha (Lepidoptera, Tortricidae). -*Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam* **3**, 123-128.

CONTROL OF THE VARROA MITE BY TREATMENT OF SEALED HONEYBEE BROOD WITH FORMIC ACID

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SUMMARY

A new control method, based on formic acid treatment of *Varroa* infested worker brood outside the colony, was tested. During the emergence of bees from the treated brood, the killed mites fell to the bottom of the hive, where they were counted. When all the bees had emerged from the treated brood, the number of mites left in the colonies was assessed. The percentage of mites fallen out of the treated brood cells was 73.5 % and 80.6% of the mites recovered from the colonies, for two variants of the control method.

Mites killed in brood cells by formic acid, were found at the bottom of the hive after emergence of the bees from the treated brood. Since brood cell invasion starts just before capping, mites invade into and emerge from brood cells in a similar sequence. The rate of invasion of mites into brood cells is related to the ratio between the number of capped brood cells and the colony size. A prediction of the number of mites invading brood cells and, thus, falling to the bottom of the hive after emergence from the treated brood, in the course of the experiment, was made. The number of mites counted at the bottom of the hive was compared with this prediction.

The effect of the control method could be predicted for a group of colonies of known size and number of capped brood cells. Using the model developed for the prediction of the effect, conditions for an optimal variant of the control method are given.

INTRODUCTION

The Varroa mite, *Varroa jacobsoni* Oud., parasitizes the honey bee, *Apis mellifera* L. (Ritter, 1981; de Jong, 1984; Ifantidis & Rozenkranz, 1987). Adult female mites invade brood cells just before capping (Boot *et al.*, 1991). Reproduction takes place inside the capped brood cell. When the bee larva has developed into an adult, the mites and their offspring leave the brood cell together with the emerging bee. Parasitization of bees causes malformations and a shortened life span (Beetsma *et al.*, 1989). Without control measures bee colonies perish within a few years after infestation (Ritter, 1981).

Treatment of honeybee colonies with formic acid for control of *Varroa* mites, is effective (Ritter & Ruttner, 1980) and environmentally safe since formic acid naturally occurs in honeybee colonies (Crane, 1975). However, major disadvantages of formic acid treatment of colonies are the damage to uncapped brood and young bees and the loss of queens in a small percentage of the colonies (Liebig, 1984; Calis *et al.*, unpublished).

Mites inside capped brood cells are trapped and can therefore easily be removed from a colony. This principle is used in biotechnical mite control methods (Maul, 1983; Maul & Klepsch, 1987). A large part of the mites is trapped in a few combs, which are destroyed subsequently. A major disadvantage of these methods is the destruction of bee brood. Fries (1991) demonstrated that formic acid treatment of capped brood combs outside the colony in sealed plastic foam boxes can effectively kill the trapped mites. The brood was not damaged except for pupating brood resulting in a total brood mortality of about 5 %. A combination of a trapping comb technique and a formic acid treatment of the

capped brood may be an improvement because the treated brood can be returned to the colonies.

The aim of this study was to design and test a new control method that combines trapping combs with formic acid treatment of these combs. The effect of such a method largely depends on the percentage of mites that invade the trapping combs. The rate of invasion of mites into brood cells is related to the ratio between the number of capped brood cells and the colony size (Calis *et al.*1991). Mites killed in brood cells by formic acid, can be found at the bottom of the hive after emergence of the bees from the treated brood. Since brood cell invasion starts just before capping, mites invade into and emerge from brood cells in a similar sequence. Therefore, a prediction of the relative number of mites invading brood cells and, thus, falling to the bottom of the hive from treated brood, can be made when colony size and the number of capped brood cells is assessed. When the effect of the control method can be predicted, the model used for this prediction can be used for the evaluation and optimization of the presented control method and other control methods based on trapping combs.

MATERIAL AND METHODS

Honey bees of hybrid origin were used. The colonies were kept in hives, type *spaarkast*, in two 10-frame brood chambers. The colonies were infested with *Varroa* mites by introduction of brood from heavily infested colonies. The experiment was conducted in 1991.

Obtaining brood for treatment.

Two batches of capped brood, 9 to 18 days old, were treated with formic acid. To obtain the dated brood, we placed the queen, on August 1, in the upper chamber above a queen excluder. After 9 days, on August 10, the queen was placed in the other chamber. Again 9 days later, on August 19, the brood produced by the queen in the first chamber was of the proper age and was treated with formic acid. After treatment, the queen was placed in the chamber with the treated combs. The brood in the second chamber was similarly treated on August 28, after which the queen excluder was taken away (see figure 1).

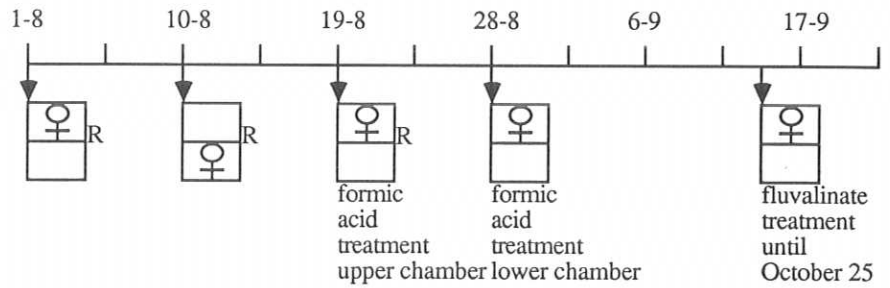

Figure 1. Schematic representation of the Varroa *control method using formic acid treatment of capped brood outside the colony. The date is shown on the top line starting on August 1, 1991. Indicated is the location of the queen. The squares represent chambers of a hive. R= queen excluder.*

Two variants

Formic acid treatment of original brood was prevented differently in two variants. In ten colonies the experiment was started by confining queens in the upper brood chamber with no uncapped brood (variant 1). In ten other colonies the queen was first confined to a queen cage for four days from July 28 to August 1 (variant 2). In this way,

the effect of a 4 days shortened emergence period of the original brood on the trapping of the mites could be observed.

Treatment with formic acid

The formic acid treatment developed by de Ruijter *et al.* (1990) was used. Ten combs were placed in a box made of extruded polystyrene foam parts fixed to each other with polyurethane glue. Formic acid, 30 ml 85%, was applied to 5 beer spills placed on top of the combs and subsequently the box was sealed for one hour. After treatment the combs were returned to their original colony. The effect of the formic acid treatment was assessed after 2 two days by examining about 100 mites from treated worker brood cells. No surviving mites were found in worker brood cells.

Assessment of the effect of the method

Mites killed in treated brood fell to the bottom of the hive when the bees emerged from the brood cells. Every two or three days the killed mites were collected from the bottom board of the colony. When all the formic acid treated brood had emerged and, thus, the killed mites had fallen to the bottom board, strips, containing fluvalinate, were placed in the colonies from September 13 to October 25 to kill the mites left. The effect of the method was the percentage of mites found on the bottom board before fluvalinate treatment of the total number of mites found on the bottom board.

Assessment of colony weight and the number of brood cells

The weight of the colonies was measured by assessing the difference in weight of a hive with bees and without bees. On the evening of August 27 all hive entrances were closed. In the morning of August 28 all hives were weighed. Subsequently the bees were temporarily removed until the hives were weighed again. The number of treated brood cells for each colony was estimated using a grid divided into areas corresponding with 100 worker cells. This was done just before the formic acid treatment.

Prediction of mites found on the bottom board

The percentage of mites falling to the bottom board in the course of the experiment was predicted using the following assumptions.

1. Mites emerge equally divided over time from the original brood, that was present at the start of trapping of the mites (a period of 13 days in the first variant and a period of 9 days in the variant where the queen was confined in a queen cage for four days before the start of the experiment).

2. Mites on bees invade brood cells with a rate calculated as follows:

$r = 0.54 * Br/C$

where
r = relative invasion rate (1/day)
Br = number of available brood cells (number of cells/day)
C = colony size (grammes of bees)
0.54 = constant (Calis *et al.*, 1991 ; grammes of bees/number of cells)

The fraction of mites on the bees invading brood each day was calculated from

$Fr = \exp(-r*\Delta t)$

where
Fr = fraction of mites on the bees invading brood cells each day (1/day)
Δt = 1 day

3. Mites invade and emerge from brood cells in a similar sequence. The predicted relative number of mites invaded on one day is, after formic acid treatment of the capped brood, assumed to be found 12 days later on the bottom board.

4. Mites left on the bees after the second formic acid treatment, will be invading new untreated brood cells until the start of the fluvalinate treatment. It is assumed that each mite in this untreated brood produces one daughter. From the start of the fluvalinate treatment it is assumed that all mites on bees are killed before they can invade a new worker brood cell.

The distribution of mites over bees and original brood at the start of the trapping of the mites was unknown. The fraction of the mites found at the bottom of the hives in the course of the experiment was predicted for the extreme situations assuming all mites present on the bees or all mites in the original brood cells. By fitting the predicted fraction of trapped mites to the fraction counted on the bottom board, the original distribution of mites over bees and brood was estimated.

RESULTS AND DISCUSSION

Table 1 summarizes the data obtained from ten colonies per variant. All data from the two variants have been statistically tested using Wilcoxon's test. Only the percentage of mites trapped in the first treated brood was significantly higher ($P<0.01$) in variant 2, where the queen was confined in a queen cage. From 4 days before mites are trapped no brood is available for mite invasion. Therefore,during this 4 days, the fraction of mites present on the bees can only increase. Moreover, all mites from the original brood will have emerged 4 days earlier in variant 2. Thus, more mites will be present on the bees and will be trapped in variant 2.

Table 1. The average and standard deviation per variant per colony of: the weight of the colonies, the number of mites found on the bottom board, the percentage of mites on the bottom board after the first and second formic acid treatment and the fluvalinate treatment, and the number of capped brood cells per treatment.

	VARIANT 1		VARIANT 2	
COLONY WEIGHT (g)	1797.9 ± 477.6		1889.1 ± 485.6	
NR OF MITES PER COLONY	846.3 ± 437.0		654.5 ± 278.2	
	MITES ON BOTTOM BOARD (%)	NR OF SEALED CELLS	MITES ON BOTTOM BOARD (%)	NR OF SEALED CELLS
FIRST TREATMENT AUGUST 19	40.2 ± 11.6	5820.1 ± 2170.8	60.0 ± 9.5	5187.9 ± 612.7
SECOND TREATMENT AUGUST 28	35.2 ± 6.5	4610.0 ± 1273.9	20.6 ± 8.9	4405.0 ± 2263.5
TOTAL FORMIC ACID	73.5 ± 11.1		80.6 ± 10.4	
FLUVALINATE TREATMENT	26.5 ± 11.1		19.4 ± 10.4	

It is assumed that, once a year, the population of mites should be reduced with 95 %, to maintain a harmless population density. The average effects of the two variants are 73.5 and 80.6 % respectively. Therefore, the control method does not sufficiently reduce the mite population.

Figure 2 indicates the observed cumulative percentage of mites found on the bottom boards of the colonies from the moment of emergence of bees and killed mites from the treated brood. The upper and lower lines represent the predictions of the percentage of the mites found on the bottom of the hives in the course of the experiment, assuming, at the start of trapping of the mites, the extreme situations that either all mites are on the bees or all mites are in the original brood cells. The upper lines are quite similar for both variants, since only the colony weight and the number of capped brood cells affect these lines. When, however, all mites are presumed to be in the original brood (lower line), more mites are found on the bottom board due to the treatment in variant 2. Mites have to emerge from the original brood before they can invade trapping combs. By confining the queen to a queen cage, before placing her in the first brood chamber, the period of emergence of bees and mites from the original brood was shortened by 4 days. Therefore, the number of mites on the bees and invading the brood of the trapping combs, will be higher in variant 2.

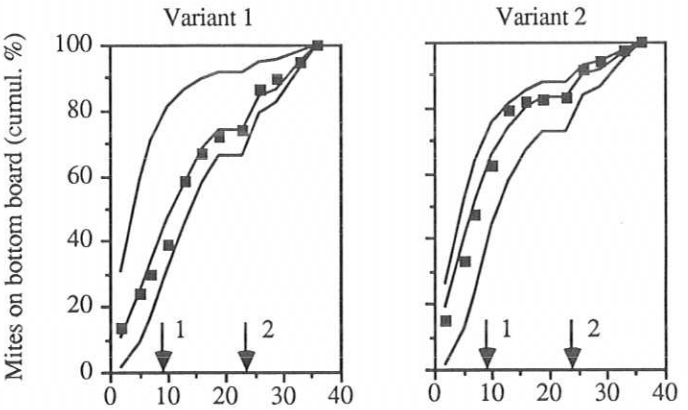

Figure 2. Cumulative relative number of mites on the bottom board from the start of emergence of bees from formic acid treated brood cells (Y-axe) versus time (X-axe). 1 = *start of emergence of second batch of treated brood,* 2 = *start of fluvalinate treatment. The upper and lower lines represent the prediction of the fraction of the mites found at the bottom of the hives in the course of the experiment for the extreme situations, assuming all mites present on the bees or all mites in the original brood cells, respectively. The dark squares represent the observed mites at the bottom board. The middle lines represent a fit of the predicted trapped mites to the observed mites at the bottom board.*

The percentages of mites found on the bottom board are within the lines that represent the predictions for the extreme distributions of the mites over bees and brood at the start of the experiment. The estimated original distribution of mites over bees and brood shows a higher percentage of the mites present on the bees for variant 2 compared with variant 1, 65% and 28% respectively. Confining the queen to a queen cage caused the absence of brood cells available for invasion of mites from 4 days before trapping of

the mites. Therefore, during these 4 days the percentage of mites present on the bees can only become higher, in variant 2, due to emergence of mites from original brood.

The estimated original distributions of mites over bees and brood are beyond the extreme distributions in 40 % of the individual colonies. The model appearently cannot accurately predict the relative number of trapped mites in individual colonies in one apiary. The assumptions used for the predictions may not be true for individual colonies. Mites in the original brood may not be equally distributed. Also, the relation between the relative invasion rate and the brood/colony-size ratio may vary between or even in colonies. The assessed weight of the colonies is only an estimate of the number of bees. Moreover, the weight of the colony was assessed only once in these long lasting experiment. In colonies with a relatively high number of mites, generally a high effect of the control method was found, while in colonies with a low number of mites, a low effect was found. This indicates that bees drifting from one colony to another during the experiment may have transferred mites.

The effect of the two variants of the control method could be predicted for a group of colonies. This demonstrates that the model can be used to evaluate and optimize *Varroa* control methods based on trapping combs. The conditions for a successful variant of this control method can, thus, be given. Formic acid treatment of a third batch of brood, similar to the second batch, would have raised the effect of both variants of the control method above 95%.

REFERENCES

BEETSMA, J., R. DE VRIES, B. EMAMI YEGANEH, M. EMAMI TABRIZI & V. BANDPAY, 1989. Effects of *Varroa jacobsoni* Oud. on colony development, workerbee weight and longevity and brood mortality. In: R. Cavalloro (ed.). Present status of Varroatosis in Europe and progress in the *Varroa* mite control, Commision of the European Communities, Luxembourg: 163-170.

BOOT, W.J., J.N.M. CALIS & J. BEETSMA, 1991. Invasion of *Varroa* mites into honeybee brood cells; When do brood cells attract *Varroa* mites? Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam 2: 154-156.

CALIS, J.N.M., W.J. BOOT, J. BEETSMA & D.J.A. SISSELAAR, 1991. How long do *Varroa* mites stay on adult bees before invading a new worker brood cell? *Med. Fac. Landbouww. Rijksuniv.* Gent, 56/2a: 313-317.

CRANE, E., 1975. Honey- a comprehensive survey. London : Heinemann

FRIES, I., 1991. Treatment of sealed honeybee brood with formic acid for control of *Varroa jacobsoni*. *Apic. Research*, May 1991: 313-314.

IFANTIDIS, M.D. & P. ROZENKRANZ, 1987. Reproduktion der Bienenmilbe *Varroa jacobsoni* (Acarina: Varroidae). Entomol. Gener. 14: 111-122.

JONG, D. de, 1984. Current knowledge and open questions concerning reproduction in the honey bee mite *Varroa jacobsoni*. In: W. Engels et al. (eds). Advances in invertabrate reproduction 3: 547-552.

LIEBIG, G., 1984. Varroa-leitfaden. Landesverb. Würtemberg. Imker, Stuttgart.

MAUL, V. & A. KLEPSCH, 1987. Biologische Bekämpfung der Varroatose. *Hess. Landesanst. Leistungspruf. Tierz. Neu-Ulrichstein Jahresb.* 1986:90-95.

MAUL, V., 1983. Empfelungen zur Methodik der Varroa-Elimination mittels Bannwaben aus Arbeiterbrut. *Allg. Dtsch. Imkerztg.* 17:179-184.

RITTER, W. & F. RUTTNER, 1980. Neue Weg in der Behandlung der Varroatose. *Allg. Dtsch. Imkerztg.* 14: 151-157.

RITTER, W., 1981. Varroa disease of the honeybee *Apis mellifera*. Bee World 62: 141-153.

RUIJTER, A., J. VAN DEN EIJNDE & D.J. DE BROUWER, 1990. Treatment of sealed brood with formic acid and the effect on the *Varroa* infestation of honeybee colonies. Report Ambrosiushoeve. November 1990.

PRODUCTION AND STORAGE OF SMALL HONEY BEE COLONIES FOR POLLINATION IN SEED CROPS

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Key words: **Apis mellifera** L, nucleus, pollination, indoor wintering

Summary

For pollination of seed crops during winter/early spring, small queenright honey bee colonies are needed. To have small colonies (nuclei) available in this period, a method has been developed. This method consists of four parts: queenbreeding, rearing of small colonies, storage of the colonies and the use of the colonies for pollination.

Queenbreeding: By queenbreeding in queenright colonies, it is possible to breed queens during an extended period with a limited number of nurse colonies.

Rearing of small colonies: Because of the extended period in which queens are bred, many mating nuclei can be composed from a limited number of colonies. Six to seven weeks after composition of the mating nuclei, they have turned into small colonies which are suitable for pollination of seed crops in small compartments in greenhouses.

Storage: During winter the small colonies can be stored at 5°C for 16 to 20 weeks.

Use for pollination: After storage the colonies can be used for pollination of seed crops in small compartments in greenhouses. The colonies remain active for at least 6 weeks.

INTRODUCTION

In co-operation with the Dutch Horticulture Seed Firms, a research programm was started to develop a method for the production and storage of small honey bee colonies. These colonies are needed for pollination of seed crops in small compartments in green houses during winter/early spring. For pollination purposes under these circumstances small queenright colonies are needed. By means of grafting larvae and queenbreeding in queenright colonies, queens can be bred with a limited number of nurse colonies during an extended period. Subsequently many mating nuclei can be made out of a limited number of colonies. As it is not possible to rear colonies during winter, queen breeding and rearing of small colonies must be done during summer. The small colonies must be stored to be available for pollination during winter/early spring. As it is known that small colonies can not survive during winter if they are stored outside, storage in a cold storage container at 5°C was tested.

MATERIAL AND METHODS

Queen breeding: For grafting larvae of about one day old, which floated in the larval food and were not yet curved to a C were, used. Grafted larvae were offered twice to the same starter hive with an interval of 24 hours. The accepted larvae were transferred to nurse colonies 24 hours after having been placed in the starter hive. Subsequently the bees of the starter hive were placed back in the colony they originated from. Four hours after the nurse colonies had been prepared (queen and sealed brood under an excluder and frames containing open brood above the excluder), the cells from the starter hives were placed between the frames containing open brood. After 4 to 5 days, the queen cells were sealed. At that time the nurse colonies were rearranged again to obtain the same start position. Some hours later on new accepted cells from starter hives were placed in the nurse colonies. Ten to eleven days after the larvae were grafted, the sealed queen cells were placed in queen cages. The queens emerged in these cages in the nurse colonies. Accepted larvae from starter hives were placed every 4 to 5 days in the nurse colonies to be reared.

Mating nuclei were composed with young bees. The bees were placed in a Kirchhainer hive. A Kirchhainer hive is a styrofoam trapezoid hive. The inner measures of the hive are: upper side 16 x 21.5 cm, bottom 10.5 x 21.5 cm, depth 10.8 cm. The hive contains 6 top bars at which the bees build combs. The combs are about 1 dm^2. The combs remain unattached from the inner surface of the hive. Half an hour after the mating nuclei were composed, the queen was introduced through the entrance. The nuclei were stored for 3 to 4 days and then placed in the mating yard. The distance between the mating nuclei was 5 meters.

Rearing of the small colonies: After the mating nuclei were placed the bees were fed weekly with sugar dough. During August/September the colonies were prepared for storage by feeding them 2750 ml Api-Invert, an invert sugar solution containing 72.7% sugar. In this sugar solution 1 g Fumidil B / litre solution was dissolved to suppress Nosema disease.

Storage: In the first week of November the small colonies were placed in a cold storage container at 5° C. The hives were weighed every 2 weeks. When they reached a minimum weight, they were fed. In January, when there was a warm day, the colonies were placed outside for some hours to defecate.

Use for pollination: The colonies were placed directly from the cold storage container into the greenhouses. Activity and pollen collection (pollination activity) were recorded.

RESULTS AND DISCUSSION

Queen breeding: Each starter hive accepted on average 40 larvae. The acceptance rate of larvae, placed in the starter hive 24 hours after the first larvae, exceeded the acceptance rate of the first larvae. In the nurse colonies, 12 queens were reared at the maximum each time larvae were offered. The nurse colonies could be used 5 times in a sequence of 5 days. The majority of the queens emerged 13 to 14 days after grafting. The emerged queens can be stored in the nurse colonies for a maximum of 24 hours in case sugar dough is available in the queen cages.

Rearing of the small colonies: To fill a mating nucleus, bees covering one comb (outer measures:38 cm x 21 cm) must be taken from normal sized healthy colonies. With intervals of 5 days, from such a colony the amount of bees covering 9 to 10 frames can be taken without harming the colony. In about 80% of the mating nuclei the queen started a brood nest. Six to seven weeks after composition of the mating nuclei they

contained 6 combs covered with bees and containing brood and food. They have turned into small colonies. The colonies in the well populated Kirchhainer hives maintained this size during the rest of the summer until storage. The mean consumption of sugar dough during rearing was 100 g/week.

Storage: It turned out to be possible to store small colonies at 5°C for at least 16 weeks without increasing mortality and for a maximum period of 20 weeks. The flight entrance of the hives must be kept open on order to dispose of crumbs of wax and dead bees. During storage the bees kept walking around actively, so in front of the flight entrance a limited closed room must be created. Colonies which ran out of food could be fed with sugar dough in the cold storage container.

Use for pollination: After storage the colonies in Kirchhainer hives, which contain enough bees to cover at least 4 combs can be used for pollination in small compartments in greenhouses. It takes about a week before the bees start to collect pollen. The colonies remain active for at least 6 weeks.

REFERENCES

MC CUTCHEON, D.M., 1984 - Indoor wintering of hives. *Bee World* **65** (1), 19-37

HARNAJ, V., 1980 - Königinnezucht, biologische Grundlagen und technische Anleitungen. *Apimondia Monographien. Apimondia Verlag Bukarest.*

MAUL, V. & H. SCHNEIDER, 1985 - Überwinterung von Reservekönginnen in Kernvölkchen im Dunkelraum. *Jahresbericht Hessische Landesanstalt für Leistungsprüfungen in der Tierzucht*, 64-65.

RUTTNER, F., 1973 - Zuchttechnik und Zuchtauslese bei der Biene.*Ehrenwirth Verlag München* ISBN 3-431-1587-5.

VELTHUIS, H.H.W. & M.J. DUCHATEAU, 1983 Koninginneteelt. *Uitgave Verg. Fysiologie Rijksuniversiteit Utrecht.*

IMPROVING THE COUNTING OF HONEYBEES LEAVING OR ENTERING THE HIVE

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Key words: *Apis mellifera*; computerized counting

Summary

A device was developed to registrate the time at which each bee enters or leaves the entrance of a hive and the duration of this act. This diminishes counting errors common to this type of devices.

INTRODUCTION

The recording of the number of bees passing the entrance of a hive can be used to measure mortality in the field or compare different stocks when these are subjected to different experimental conditions.

In this study a device is presented which counts bees going in and out of the entrance of a beehive. Several of these devices have been reported in literature (Erickson 1975) but those suitable for beehives do not pay attention to the behaviour of bees in the entrance. This negligence can lead to an overestimation of the amount of bees passing the entrance.

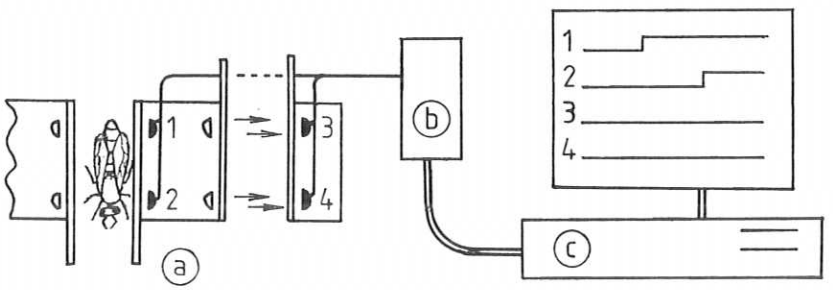

fig 1. ***Schematic representation of the bee counter.***
a) part of the entrance block with a bee in one of the glass tubes.
b) amplifiers.
c) computer with the state of the LED's on screen.

MATERIAL & METHODS

The data gathering apparatus consisted of a special entrance block fitted to a six comb British Standard hive, amplifiers and a computer as a recording device. The entrance block consisted of four glass tubes of 9 mm diameter, fitted in a block of black plastic (see fig 1). In the plastic block two infrared light emitting diodes and two light sensitive diodes were fitted for each glass tube. Thus, a passing bee disrupts two lichtbeams while entering or leaving the hive. The state of the light sensitive diodes was logged on the disk of a personal computer.

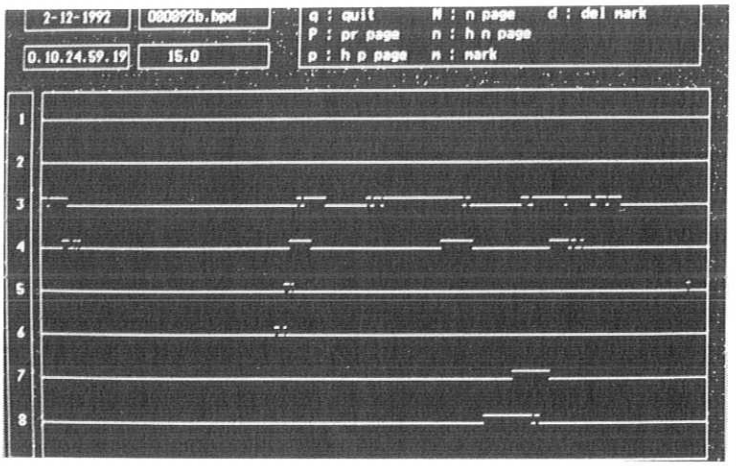

fig 2. Photo of the output of 8 channels of the entrance block.
Channels 1 & 2, 3 & 4, 5 & 6, 7 & 8 belong to one glass tube. The duration of the registration is 15 sec.

RESULTS & DISCUSSION

An example of the passage of several bees passing through the entrance block is shown in fig 2. The bees often hesitate upon entering or leaving the glass tubes (see channel 3 & 4). This sort of behaviour is also observed in *Vespula* spec. (Visscher 1983). As a consequence of this behaviour the amount of bees passing the entrance will be overestimated if only one lightbeam is fitted or the duration of the act is not recorded.

REFERENCES

Erickson, E.H., H.H Miller, D.J. Sikkema, 1975 - A method of separating and monitoring honeybee flight activity at the hive entrance. Jour. Apicult. Res. 14(3/4), 119-125.

Visscher, P.K., K.G. Ross, 1983 - Photoelectronic monitoring system for nesting hymenoptera. J. Kansas Entomol. Soc.56(3), 617-620.

BIOLOGICAL CONTROL OF WESTERN FLOWER THRIPS IN GREENHOUSE SWEET PEPPERS USING NON-DIAPAUSING PREDATORY MITES

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Key words: *Frankliniella occidentalis, Amblyseius cucumeris, Amblyseius degenerans,* biological control, diapause

Summary

The Naaldwijk strain of the predatory mite, *Amblyseius cucumeris* (Oudemans), is successfully used for biological control of thrips in greenhouses, but it appears not very effective in greenhouse crops during winter. This is probably because the females of the predator species respond to short-day photoperiods by entering diapause. Another cause may be low humidity which negatively affects hatching success of predator eggs. A search for predators that do not enter diapause and/or that are less sensitive to low humidities, resulted in two phytoseiid species as candidates for use in biological control of thrips in greenhouses: (1) a strain of *A. cucumeris* with a low incidence of diapause, and (2) a drought-resistant non-diapausing strain of *Amblyseius degenerans* Berlese.

In the present study these two strains and the Naaldwijk strain of *A. cucumeris* are compared with respect to their performance as biological control agent of western flower thrips in a sweet pepper crop during winter. The results of greenhouse experiments in the period of January to September showed that the establishment early in the season (January, February, March) was greatly improved when using the two non-diapausing strains. The impact on thrips populations during the growing season was greater for *A. degenerans* than for the strains of *A. cucumeris*.

INTRODUCTION

Western Flower Thrips, *Frankliniella occidentalis* (Pergande), is an important pest of greenhouse-grown sweet pepper. In Dutch greenhouses this thrips pest is biologically controlled by the predatory mite, *Amblyseius cucumeris* (Oudemans). Starting from March the predator is released successfully as its population persists in the crop, making reintroductions superfluous. This persistence may be attributed to the availability of pollen as an alternative food source (Van Rijn & Sabelis, 1992). Consequently, the predators may even be introduced before thrips infestation. During winter, however, introductions of *A. cucumeris* are not successful, which may be caused by the

occurrence of diapause (Morewood & Gilkeson, 1990; Van Houten, 1991) and the insufficient resistance of the predator's eggs to low air humidity, commonly occurring during frost periods. Thus, a non-diapausing thrips predator that is tolerant to low humidities could be a better control agent.

In search of such a control agent, Van Houten et al. (1992) compared 6 species of phytoseiid predators of thrips with respect to a number of relevant characteristics, viz. (1) predation and oviposition rate on a diet of thrips larvae, (2) diapause incidence under short-day conditions, (3) egg hatching success at low humidities and (4) rate of oviposition on sweet pepper pollen. The results of this study showed that a Mediterranean strain of *Amblyseius degenerans* Berlese may be a good candidate. This predator does not enter diapause and is more tolerant to drought than *A. cucumeris*. In another study, Van Houten et al. (in prep) collected a strain of *A. cucumeris* in New Zealand. They selected a line from this strain that showed a diapause response of 5% under short-day conditions.

The present study was undertaken to evaluate the performance of a non-diapause and/or drought-resistant predator as biocontrol agent of thrips. Therefore, *A. degenerans* and two strains of *A. cucumeris* (New Zealand and Naaldwijk) were compared with respect to (1) their establishment early in the growing season (January, February, March) and (2) their establishment and efficacy in controlling Western Flower Thrips during the growing season.

MATERIALS AND METHODS

Amblyseius degenerans originated from an insectary culture of the University of California, Riverside, U.S.A. The Naaldwijk strain of *A. cucumeris* was obtained from the Glasshouse Crops Research Station in Naaldwijk, The Netherlands. The other strain of *A. cucumeris*, with a low incidence of diapause, was collected near Auckland, New Zealand. All species were mass-reared on plastic rearing units ('arenas') as described by Overmeer et al. (1982). Pollen of the broad bean, *Vicia faba* L., was used as a food source.

The experiment was carried out in a small greenhouse with 12 rows of 15 sweet pepper plants, at the Glasshouse Crops Research Station in Naaldwijk. Each strain was released in 4 different rows of plants. In the second week of January, 1992, when the plants had started to flower, 25 female predators were introduced per plant. At the same time a few thousand adult thrips were released to obtain a thrips infestation.

To monitor thrips and predator populations, samples of in total 15 flowers and 30 leaves from the upper part of 30 plants per treatment were taken every 2 weeks. Because there

were only few flowers in January and February, flower samples were taken starting from week 8.

RESULTS AND DISCUSSION

Establishment of the thrips predators

The New Zealand strain of *A. cucumeris* was more successful in establishment during the winter period than the Naaldwijk strain of *A. cucumeris* (Figure 1). In the first 4 weeks, the plants flowered poorly, so there was hardly any food available for the predators. Consequently the establishment of both *A. cucumeris* strains was not very successful in this period. Starting from week 5, the population of the New Zealand strain increased slowly whereas the population of the Naaldwijk strain remained at a very low level. This is an indication that application of a non-diapause strain of *A. cucumeris* may improve the establishment of *A. cucumeris* during winter in the presence of food.

The establishment of the different strains of predators during the growing season is presented in Figure 2. *Amblyseius degenerans* performed best; the predator population increased very rapidly to high densities. Until week 13 the species was only found on the release plants but from week 13 onwards *A. degenerans* was also present in the other rows where the *A. cucumeris* strains had been released. From this moment *A. degenerans* started to displace *A. cucumeris* from the crop; *A. cucumeris* populations decreased slowly and vanished completely after week 23. Starting from week 23, *Orius* spp. were noticed in the greenhouse and during the rest of the experiment *A. degenerans* and *Orius* spp. were simultaneously present in the crop.

The success of *A. degenerans* may partly be explained by the distribution of the predators on the plant. Although *A. cucumeris* and *A. degenerans* can both feed and reproduce on

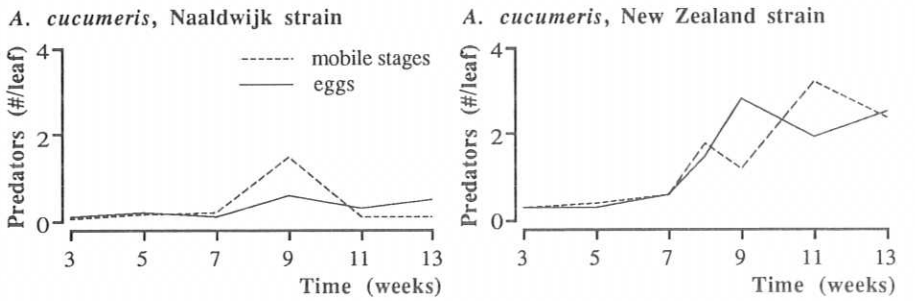

Figure 1. Population fluctuations of the Naaldwijk and the New Zealand strain of the phytoseiid mite, Amblyseius cucumeris, *on leaves of a greenhouse sweet pepper crop. In the second week of January, 25 predatory females were introduced per plant. Each strain was released in 4 different rows of plants.*

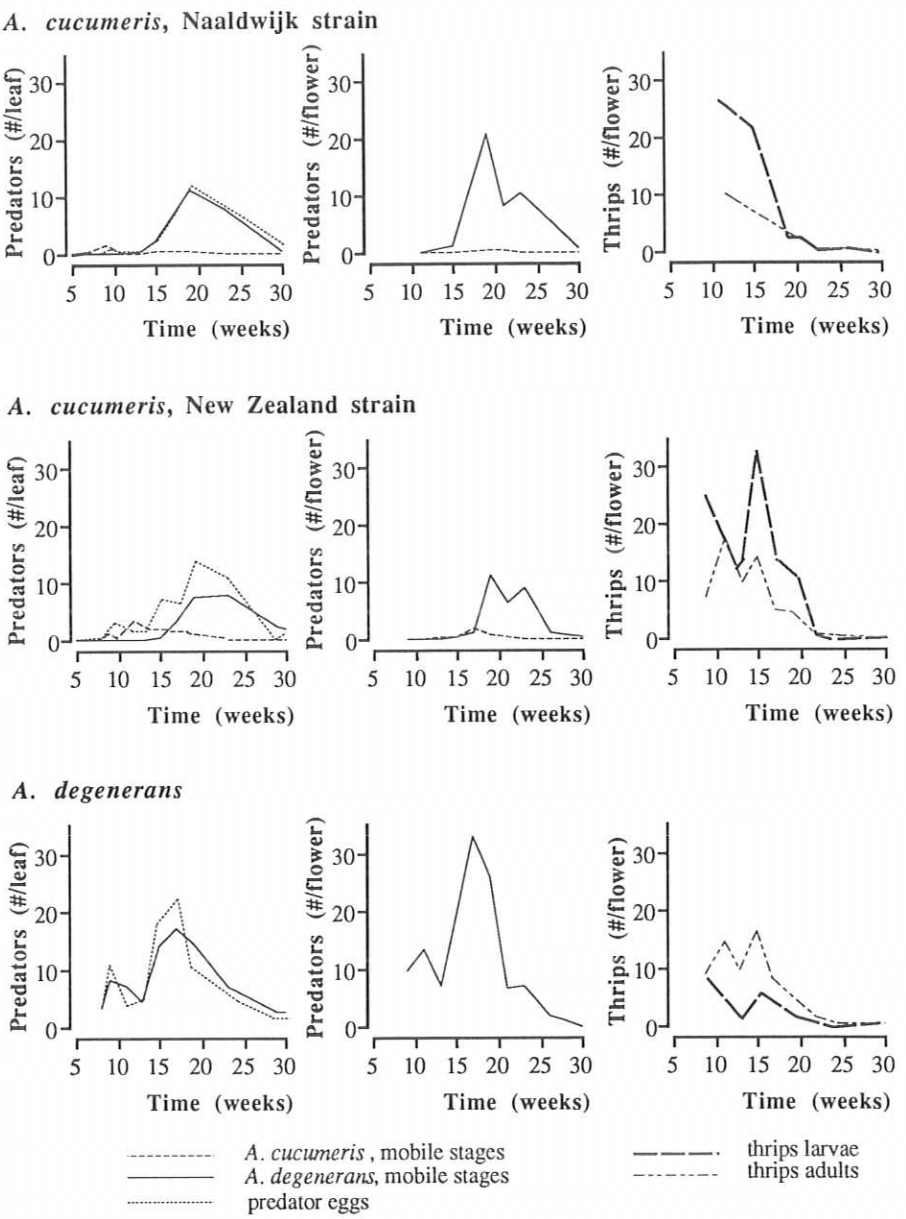

Figure 2. Population fluctuations of Western Flower Thrips, Frankliniella occidentalis, *the phytoseiid mite,* Amblyseius degenerans, *and two strains of the phytoseiid mite,* A. cucumeris, *on leaves and in flowers of a greenhouse sweet pepper crop. In the second week of January, 25 predatory females were introduced per plant. Each strain or species was released in 4 different rows of plants.*

pollen (Van Houten et al., 1992), there appears to be a difference in the rate at which they visit flowers (Van Houten, unpublished results). During the experiment the thrips density in flowers was considerably higher than the thrips density on leaves. By visiting flowers more frequently, *A. degenerans* may take better advantage of the pollen and/or thrips larvae as a food source than *A. cucumeris*. This in turn could lead to increasing establishment, survival, rate of development and rate of oviposition of *A. degenerans*. Which stimulus causes *A. degenerans* to visit flowers and why *A. cucumeris* hardly visits flowers needs to be investigated.

Another characteristic of *A. degenerans* is that eggs of this predator are more resistant to drought than the eggs of *A. cucumeris*. The winter of 1992, however, was very mild and therefore it was assumed that the humidity levels in the greenhouse have not been below the critical level for the eggs of *A. cucumeris*.

Impact of the predators on thrips populations

Unfortunately, our results do not allow to conclude whether the New Zealand strain of *A. cucumeris* is better able to control thrips than the Naaldwijk strain. This is because only three flower samples had been taken before *A. degenerans* invaded the plant rows with *A. cucumeris* (Figure 2). The results do show that *A. degenerans* decimates thrips populations. *Amblyseius degenerans* wipes out the thrips not only in plant rows where it originally was released, but eventually in all other plant rows in the greenhouse.

It remains to be shown which characteristics enable *A. degenerans* to control thrips. Laboratory experiments revealed that the rate of predation and oviposition on a diet of thrips larvae is somewhat lower for *A. degenerans* than for *A. cucumeris* (Van Houten et al., 1992). Therefore, successful thrips control by *A. degenerans* has to be explained by other factors. One of these may be searching efficiency of the predator. We observed that *A. degenerans* shows higher locomotory activity on the leaves than *A. cucumeris*. Moreover, unlike *A. cucumeris*, which is predominantly found on leaves, *A. degenerans* is also found in substantial numbers in flowers, where there is a higher thrips density (see Figure 2). Laboratory experiments have shown that both predators feed on thrips larvae when pollen and thrips are offered (Van Houten, unpublished results). Both higher locomotory activity and the frequent presence in flowers could lead to a higher number of encounters with thrips larvae, and hence to higher thrips mortality. Experiments at the individual level are needed to elucidate the underlying mechanism.

Future research will show whether or not *A. degenerans* is a good candidate for thrips control in other greenhouse crops as well. A good mass-rearing technique for *A. degenerans* has still to be developed.

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REFERENCES

HOUTEN, Y.M. VAN, 1991. Diapause induction in the thrips predators *Amblyseius barkeri* and *Amblyseius cucumeris* (Acari: Phytoseiidae) in Dutch greenhouses. Proc. Exp. & Appl. Entomol., N.E.V. Amsterdam **2**: 202-207.

HOUTEN, Y.M. VAN, VAN RIJN, P.C.J., TANIGOSHI L.K. & STRATUM, P. VAN, 1992. Potential of some phytoseiid species to control *Frankliniella occidentalis* in greenhouse crops. IOBC/WPRS Bulletin (in press).

MOREWOOD, W.D. & GILKESON, L.A., 1990. Diapause induction in the thrips predator *Amblyseius cucumeris* (Acarina: Phytoseiidae) under greenhouse conditions. Entomophaga **36**: 253-263.

OVERMEER, W.P.J., DOODEMAN, M. & VAN ZON, A.Q., 1982. Copulation and egg production in *Amblyseius potentillae* and *Typhlodromus pyri* (Acari, Phytoseiidae). Z. angew. Entomol. **93**: 1-11.

RIJN, P.C.J. VAN & SABELIS, M.W., 1992. Does alternative food always enhance biological control? The effect of pollen on the interaction between Western Flower Thrips and its predators. IOBC/WPRS Bulletin (in press).

IPM IN TEA: THE CASE OF PREDATORS AGAINST SCARLET MITES

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Key words: *Brevipalpus phoenicis,* Phytoseiidae, Stigmaeidae, biological control, *Camellia sinensis*

Summary

Possibilities for Integrated Mite Control in tea were investigated. Several native predatory mite species were found in the field. These were succesfully reared in the laboratory. Mass rearing was attempted using alternative food sources. The predators susceptibility to the pesticides commonly used in tea should be investigated.

INTRODUCTION

The scarlet mite, *Brevipalpus phoenicis* Geijskes, is the most damaging phytophagous mite on tea in Indonesia. Although it can be found everywhere in the plantations, the damage it causes is restricted to specific areas only. The presence of some native predatory mites (Fam. Phytoseiidae and Stigmaeidae) and their effect on the pest have been studied by Oomen (1982), who ascribed most importance to the Stigmaeidae. Our present aim is to establish a basis for applying augmentative releases of predatory mites.

MATERIALS AND METHODS

The Gambung tea plantation (Research Institute for Tea and Cinchona), 45 km South of Bandung, West Java, Indonesia, was explored for Stigmaeid and Phytoseiid predators by taking 50 tea leaves from each of 20 individual blocks of tea bushes in diagonal transsects. Found predators were immediately transferred to starter cultures using the modified MacMurtry&Scriven method. The predators were then given the following food sources: Tetranychid mite eggs, Scarlet mites from the field, pollen collected from tea, poinsettia, broadbean and iceplant.

RESULTS AND DISCUSSION

On the leaf surface Phytoseiidae are more important than Stigmaeidae. Apart from Phytoseiidae and Stigmaeidae listed in Table 1 many other arthropod taxa were found in the samples, some of which can serve as food for the predators when scarlet mite density is low. They included:
Tetranychidae, Eriophyidae , Acaridae, Ascidae, Tydaeidae, Tarsonemidae, Oribatidae, Bdellidae, Cheyletidae, Thrips and Collembola.

Table 1. Predatory mites found on leaf samples at Gambung, Indonesia

Family / species	host plants	laboratory rearing
Phytoseiidae		
Amblyseius deleoni	tea, weeds	good, reas. fast
Phytoseius crinitus	tea weeds	good, slow
Amblyseius quadridens	tea, weeds	not yet
Proprioseiopsis euflagellatus	tea	difficult
Species 5, not yet identified	weeds	good, very slow
Typhlodromus jackmickleyi	tea	good
Species 7, not yet identified	tea	not yet
Neoseiulus sp	tea,weeds	reasonable
Stigmaeidae		
Zetzelia javanica	tea	not yet
Agistemus orbicularis	tea	not yet

Several predator species were succesfully reared using the MacMurtry & Scriven method. *A. deleoni* could thus be reared on scarlet mites, tetranychid eggs and pollen. Development from egg to adult took 8-9 days at 25° C. This species is dominant in the field, and in tea plantations which are undisturbed by pesticides it seems to be controlling scarlet mite. Therefore it may be a suitable candidate for resistance selection, mass rearing and augmentative releases. The predator's susceptibility to the common pesticides used on tea should be further investigated.

Integrated mite management must become part of a total IPM package for the tea crop.

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REFERENCE

OOMEN, P. A. (1982) - Studies on population dynamics of the scarlet mite, *Brevipalpus phoenicis*, a pest of tea in Indonesia. Meded. Landb. Hogeschool Wageningen No. 82-1

LATHROLESTES ENSATOR, A PARASITOID OF THE APPLE SAWFLY

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Key words: *Hoplocampa testudinea, Lathrolestes ensator*, apple sawfly, orchard IPM

Summary

The present knowledge on the ichneumonid wasp *Lathrolestes ensator* (Brauns) is summarized. The species parasitizes larvae of the sawfly *Hoplocampa testudinea* (Klug). At higher parasitism levels superparasitism occurs. The black, comma-shaped parasitoid eggs hatch after the host larva has spun its cocoon in soil. From September until the following spring, only the *L. ensator* prepupa, having killed its host, is found in the cocoon, gradually developing into an adult, which emerges in May/June. Some prepupae have a prolonged diapause and emerge after 2 winters.

INTRODUCTION

In about 50 % of Dutch apple orchards, integrated pest management is applied, involving natural enemies and the use of selective pesticides to control perennial pests like caterpillars and aphids (Blommers, 1993). However, for several occasional pests, only organophosphates and carbamates are available for control. These broad-spectrum pesticides may disrupt the natural control of apple pests. One of the occasional pests is the apple sawfly, *Hoplocampa testudinea* (Klug) (Tenthredinidae). Last year we started to investigate possibilities of promoting parasitic wasps to control this pest. Here we present some preliminary data.

THE APPLE SAWFLY

Geographical distribution

H. testudinea is widely distributed over Europe (Commonwealth Agricultural Bureaux, 1964), roughly between 40 and 60 degrees North latitude (Velbinger, 1939). Outside Europe it is known in northern Turkey and western parts of the former USSR. In the USA it was accidentally introduced in 1939 at Long Island, New York, and has spread across the entire north-east. In Canada, for almost 30 years it was restricted to Vancouver Island, British Columbia, where it was first observed in 1940. However, since 1979 it is also found in Quebec (Paradis, 1980).

The biology of the apple sawfly

The apple sawfly *H. testudinea* was first thoroughly studied by Miles (1932) and Velbinger (1939). The species only oviposits on apple and is univoltine. It spends most

of its life in the soil, as a quiescent prepupa in a parchment-like cocoon from June to the following spring, when the adults emerge. The period of adult flight largely coincides with the flowering of apple, usually in late April and May.

The egg is laid under the epidermis of the upper surface of the flower receptacle (Miles, 1932). After an incubation period of 1-2 weeks, the larva emerges. The first instar tunnels just under the surface, leaving a scar, the second one goes deeper. The third instar migrates to another fruitlet, and tunnels directly to the developing seeds. A third apple can be penetrated, before the full grown fifth instar larva leaves the fruit to enter the soil by the middle of June. In the soil a cocoon is constructed and the prepupa starts diapause. A developed pupa is not found until about 3-4 weeks prior to emergence (Miles, 1932). In experiments at the Schuilenburg development into a pupa became apparent between 12th-19th March 1991 and 9th-27th March 1992.

Some prepupae have a prolonged diapause, and don't develop into a pupa and adult until after the second year (Kuenen & Van de Vrie, 1951). Experiments in 1977 and 1978 by W. R. Simons & P. Gruys (unpublished, see below) showed that one year after 250 larvae had entered the soil 26% and 17% respectively emerged as an adult, and after two years (prolonged diapause) 9% and 1% emerged. In one case after three years two adults emerged.

Visual traps (Rebell™), white sticky plates that don't reflect ultraviolet, can be used to monitor the flight of the sawfly (Haalboom, 1983). During 1985-1992 flight periods were assessed on trees of cv. Summerred. The day that the first sawfly was trapped varied from 16th April-9th May, the last from 14th May-5th June; an average period of 26 days.

Rearing of *H. testudinea*

At the Schuilenburg we obtain sawflies for experiments by collecting apples with full grown larvae. The fruitlets are placed on wire netting above a bucket, and the descending larvae are transferred into open plastic pots with sandy soil, mostly twice daily, which are placed outdoors. They usually burry themselves immediately and make a cocoon.

The success rate of such rearings is quite variable, as various fungus diseases may kill the sawfly under ground (Jaworska, 1979a). In some of our material *Paecilomyces fumosoroseus* (Wize) Brown et Smith was identified by R. Samson, C.B.S., Baarn. On the other hand desiccation may also kill the animals. In an experiment, pots with sawfly larvae in -initially humid- soil were kept dry, or were moistened once (4.xii.91) or twice (again on 13.iii.92). Sawflies emerged from 14, 83 and 95% of the cocoons (n=43, 42 and 41) respectively.

Reported mortality agents

The probably first record about insect parasitoids of *H. testudinea* was published by Velbinger in 1939; a single specimen of *Lathrolestes ensator* (Brauns). The same parasitoid was reported from apple sawfly in the Baltic Sea area by Cakstynja in 1968 (in Jaworska 1987). In Moldavia, Talickiej (1966)(in Jaworska 1987) reared two other species from sawfly larvae: *Phygadeuon talitzkii* and *L. luteolus* (Thoms.). Some other East European references about parasitoids, reared from *H. testudinea*, can be found in Dulak-Jaworska (1976) and Jaworska (1987).

Research on the natural mortality of *H. testudinea* was done in the 1970's in

Poland. Niezborala (1976) reported that *L. citreus* Brischke parasitized 33 % of apple sawfly larvae in 1969. Dulak-Jaworska (1976) collected larvae of *H. testudinea* and reared five different species of ichneumonid wasps, 70 % of the specimens belonging to *L. marginatus* (Thompson). This species is the main parasitoid of the apple sawfly in Poland (Jaworska, 1987).

According to Jaworska (1992) entomopathogenic fungi can cause high mortalities in *H. testudinea* in the soil. Niezborala (1976) reported that in 1971 an unidentified fungus caused 97 % mortality in cocoons. In the former Soviet Union, *Paecilomyces fumosoroseus* (Wize) Brown et Smith. and *Beauveria bassiana* (Bals.) Vuill. caused 15-30% mortality under natural conditions (Onufreichik, 1974). In field experiments by Jaworska (1979a), soil treatment with 7 fungi reduced the numbers of emerging adults, *P. fumosoroseus* and *P. farinosus* being most effective. Sawflies that survived treatment with *Aspergillus flavus*, *B. bassiana* and *B. tenella*, showed a decrease in fertility and adult life span (Jaworska, 1979b). It was shown that fungi cause greater mortality among larvae, parasitized by ichneumonids, than unparasitized ones (Jaworska, 1979c). Jaworska (1987) found that mortality of *L. marginatus* larvae was higher before they had killed the host and made their own cocoons (52%), than after this moment, when larvae were more resistant (13-18% mortality). Jaworska (1987) concludes that parasitic wasps do not play an important role in reducing sawfly populations.

Dead larvae and pupae infested by nematodes were found in cocoons of apple sawfly by Jaworska (1986). Only *Heterorhabditis* sp. showed high pathogenicity to apple sawfly larvae in laboratory, and, when introduced into the soil, caused high mortalities among both sawfly larvae that were about to start diapause and larvae that have already made a cocoon (99% and 58%, respectively). In experiments by W. R. Simons & P. Gruys (unpublished) at the Schuilenburg in 1977 and 1978, plots were treated with the nematode *Neoaplectana carpocapsae* Weiser, before full grown larvae were allowed to enter the soil. Emergence of adult sawflies was followed during three years. The treatment resulted in a significant reduction (table 1).

Table 1 Average (± standard deviation) emergence of adult sawflies from plots (10 replicates) sprayed with nematode suspension and water (untreated). 25 larvae per plot had entered the soil after treatment.

	1977		1978	
treatment	nematodes	water	nematodes	water
Average ± S.D.	2.0 ± 1.2	8.7 ± 3.5	2.4 ± 2.7	4.7 ± 1.3
Emergence (%)	8	35	10	19
Effect (%)	77		57	

It should be noted that all these observations and experiments concern the survival of *H. testudinea* during the cocoon stage in the soil.

LATHROLESTES ENSATOR

In 1983, adult *Lathrolestes* sp. (Ichneumonidae Ctenopalmatinae) emerged from cocoons made by apple sawfly larvae collected in the experimental orchard in the previous year. Mr. R. Hinz from Einbeck, Germany, identified some of these specimens

as *L. ensator* (Brauns). Similar specimens have been reared since, both from this and other localities, and no other parasitoids. So far, hardly anything seems to have been reported on the biology of *L. ensator*, which is closely related, if not identical to, *L. marginatus* (R. Hinz, in litt. 1991). Both have never been reported from another host than *H. testudinea*.

At the time the sawfly larvae descend, the black comma-shaped eggs of *L. ensator* can be seen through the host skin, even with the naked eye. In 1992, we found mostly one, and up to 4 parasitoid eggs per larva, by dissection. The egg distributions in larvae found in 6 orchards with different parasitism levels (6, 12, 16, 19, 38 and 77%) were not significantly different from the Poisson distribution (chi-square test, df=4, $p>0.05$ at 77% parasitism, and $p>0.5$ for the other 5 levels). So the eggs were more or less randomly distributed over the host larvae.

The development of *L. ensator* was followed in two seasons (1990-1992) by dissecting cocoons from rearings in the outdoor insectary. About 2-3 weeks after the sawfly larva had entered the soil, the parasitoid egg had eclosed; an 0.7-0.9 mm long larva was found in its host, with a brownish head capsule and a 0.2-0.3 mm long tail, and an empty egg with the broad end broken off. By the end of September, the parasitized sawfly larva (=prepupa) had disappeared, and a parasitic prepupa of 6-7 mm was found, that had constructed a filmy cocoon against the inner wall of the host cocoon, excluding the head capsule and other remains of the host. We observed never more than one prepupa per cocoon. By November, the body of the parasitoid was slightly depressed behind the head region, and in January the brown eyes of the adult shone through the prepupal skin. Developed pupae were present in February. Apparently, *L. ensator* continues to develop through winter, without a quiescent period, in contrast to its host.

Adult *L. ensator* start to emerge 5-14 days after the end of bloom, by the time the flight of the apple sawfly is over (table 2). At this time host eggs and mining larvae are available. Parasitoid eggs are already found in the first larval instar. Unfortunately, we so far have been unable to create the right conditions for mating and egg laying by *L. ensator* in captivity.

Table 2. Day of last trap catch of apple sawfly at the experimental orchard, the first day of emergence of L. ensator in either field cages or outdoor insectary, and the end of bloom.

	Apple sawfly		*L. ensator*				
	last date	N	1st date	N		end bloom of cultivar:	
1983	1 June	69	3 June	33	field	20 May	Golden Delicious
1984	12 June	156	2 June	2	field	25 May	Golden Delicious
1986	31 May	14	10 June	3	insectary	27 May	Elstar
1989	22 May	80	22 May	5	insectary	16 May	Elstar
1990	14 May	252	6 May	25	insectary	1 May	Elstar
1991	5 June	376	18 May	50	insectary	10 May	Elstar
1992	27 May	588	20 May	11	insectary	15 May	Elstar

Prolonged diapause also occurs in *L. ensator*; development stops in the prepupal stage. This is apparent from November onward. The rates at which this "staying over" occurs appears to be rather similar in host and parasitoid, as is shown in table 3. In this case, some cocoon batches were dissected during the month before emergence of the

sawfly, and others after the emergence of the adults. Dead sawfly prepupae were included. In as far as some of these prepupae might have died early, the rate of staying over in the sawfly, and the rate of parasitism should be over- and underestimated, respectively.

Table 3. Number of prepupae (staying over) and pupae and/or adults observed in the 1990 rearing of sawfly larvae from the experimental orchard in spring 1991.

	prepupae	pupae/adult	staying over	Total
Apple sawfly	47	70	40%	117
L. ensator	50	63	44%	113
parasitism	52%	47%		49%

L. ensator appears to be present in most apple sawfly populations in IPM apple orchards in the Netherlands. Samples were taken in 14 orchards in the centre and the south in 1991 and/or 1992 and it was encountered in 12 of these orchards. The highest rates of parasitism we found, were 38% (n=64), 42% (n=24) and 77% (n=30).

Rates of parasitism in the experimental orchard, estimated by rearing as in table 3, were 14% in 1989, 44% in 1990, 32% in 1991 and 38% (by counting eggs) in 1992.

DISCUSSION AND CONCLUSIONS

L. ensator is the only parasitoid species of the apple sawfly *H. testudinea* found by us in the Netherlands. As we have worked only with more or less full grown sawfly larvae, egg or pupal parasitoids, like *Phygadeuon* sp. (Talickiej, 1966, in Jaworska, 1987) should have been missed.

Most Ichneumonidae Ctenopalmatinae are, like *L. ensator*, koinobiont endoparasitoids of sawfly larvae (Gauld & Bolton, 1988). Few *Lathrolestes* species have been studied in detail (e.g. Eichhorn & Pschorn-Walcher, 1973).

Various traits of *L. marginatus* noted by Jaworska (1987) show great resemblance to those seen in *L. ensator*: particularly host specificity, flight phenology, and superparasitism. Indeed, it will be worthwhile to clarify the taxonomic identity of both.

Usually, *L. ensator* is also present when its host occurs in Dutch IPM orchards. Apparently, its reintroduction is often not necessary when the use of broad-spectrum insecticides is discontinued.

Evidently, we are not yet able to explain the great variation in parasitism among orchards, but some possible factors may be cited. The most evident innate handicap of *L. ensator*, from the practical point of view, seems to be its apparent inability to avoid superparasitism. Therefore it is unlikely that parasitism alone could reduce the sawfly density. Also, as the development underground of *L. ensator* is so different from the one of its host, it can not be excluded that the coincidence of its flight period with the presence of the preferred host stage for egg laying, is not always the best possible. Other factors, like host-feeding on, and fungal disease of apple sawfly, might increase the relative effect of *L. ensator* on sawfly populations.

Evidently, insecticides constitute a threat to *L. ensator*, especially during its flight that starts within 1-2 weeks after flowering. Some post-bloom spraying occurs in most orchards.

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REFERENCES

BLOMMERS, L., 1993. Integrated pest management in European apple orchards. *Ann. Rev. Ent.* **39** (in press).

COMMONWEALTH AGRICULTURAL BUREAUX, 1964. *Hoplocampa testudinea* (Klug). -Commonwealth Institute of Entomology, Distribution maps of pests, series A, map 166.

DULAK-JAWORSKA, M., 1976. Gasieniczniki - Ichneumonidae, pasozyty owocnicy jablkowej - *Hoplocampa testudinea* Klug (Hymenoptera, Tenthredinidae). -*Polskie Pismo ent.* **46**: 393-400.

EICHHORN, O. & H. PSCHORN-WALCHER, 1973. The parasites of the birch leaf-mining sawfly (*Fenusa pusilla* (Lep.), Hym.: Tenthredinidae) in Central Europe. -*Techn. Bull. Commonw. Inst. biol. Contr.* **16**: 79-104.

GAULD, I. & B. BOLTON (eds.),1988. *The Hymenoptera.* -British Museum (Natural History), Oxford Univ. Press, 332 pp.

HAALBOOM, W., 1983. Observations on the apple sawfly *Hoplocampa testudinea* (Klug) with the use of visual traps. -*Med. Fac. Landbouww. Rijksuniv. Gent* **48**: 157-161

JAWORSKA, M., 1979a. 'Investigations on the possibility of reduction of European apple sawfly populations by entomophagous fungi.' -*Roczn. Nauk Roln., ser. E* **9**: 169-181.

JAWORSKA, M., 1979b. Wplyw grzybow owadobojczych na dlugosc zycia i plodnosc samic owocnicy jablkowej- *Hoplocampa testudinea* (Klug) (Hymenoptera, Tenthredinidae) -*Roczn. Nauk Roln., ser. E* **9**: 183-189.

JAWORSKA, M., 1979c. The role of some entomopathogenic fungi in reduction of european apple sawfly- *Hoplocampa testudinea* (Klug) (Hymenoptera, Tenthredinidae)-laboratory studies. -*Bull. Acad. pol. Sci., ser. Sci. biol.* **27**: 1059-1062.

JAWORSKA, M., 1986. Nicienie-pasozyty owocnicy jablkowej. -*Zesz. Probl. Post. Nauk Rol.* **323**: 179-188.

JAWORSKA, M., 1987. Obserwacje nad *Lathrolestes marginatus* (Thompson), pasozytem owocnicy jablkowej - *Hoplocampa testudinea* (Klug) (Hymenoptera, Tenthredinidae). -*Polskie Pismo ent.* **57**: 553-567.

JAWORSKA, M., 1992. Biological control of *Hoplocampa testudinea* (Klug) (Hymenoptera, Tenthredinidae). -*Acta phytopath. ent. Hung.* **27**: 311-315.

KUENEN, D.J., & M. VAN DE VRIE, 1951. Waarnemingen over de biologie en de bestrijding van de appelzaagwesp (*Hoplocampa testudinea* (Klug), Hymenopt., Tenthredinidae). -*Tijdschr. Plantenziekten* **57**: 135-157.

MILES, H.W., 1932. On the biology of the apple sawfly, *Hoplocampa testudinea* (Klug). -*Ann. appl. Biol.* **19**: 420-431.

NIEZBORALA, E., 1976. Przyczynek do poznania pasozytow owocnicy jablkowej - *Hoplocampa testudinea* (Klug) (Hym., Tenthredinidae). -*Polskie Pismo ent.* **46**: 387-391.

ONUFREICHIK, K. M., 1974. Natural limitations to the abundance of sawflies. -*Zashchita Rastenii* **9**: p. 30.

PARADIS, R. O., 1980. L'hoplocampe des pommes, *Hoplocampa testudinea* (Klug) (Hymenoptera, Tenthredinidae), au Québec. -*Phytoprotection* **61**: 26-29.

VELBINGER, H., 1939. Beitrag zur Biologie und Bekämpfung der Apfel- und Birnensägewespe. -*Gartenbauwiss.* **13**: 492-566.

COMPARATIVE SUSCEPTIBILITY TO TWO INSECTICIDES OF THE PREDATOR *CHRYSOPERLA EXTERNA* HAGEN AND THE BOLL WEEVIL *ANTHONOMUS GRANDIS* BOH. IN COTTON IN NICARAGUA

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Key words: *Chrysoperla externa, Anthonomus grandis*, comparative toxicity, insecticides, cotton, Nicaragua

Summary

Results of toxicity tests with Cypermethrin and Methyl Parathion, 2 commonly used insecticides in Nicaragua, indicate that *Chrysoperla externa*, particularly its larva, is less susceptible to the synthetic pyrethroid than the Boll weevil. Toxicity of Methyl Parathion is similar for both insect species.

INTRODUCTION

Since the early fifties cotton has been a major cash crop in Nicaragua. At the same time, cotton was heading the list of main insecticides consumers. After a serious decline in cotton production integrated pest control programmes were developed (Falcon & Smith, 1973 ; Bodan *et al*, 1979 ; Frisbie, 1983), but their lasting implementation in Nicaragua has been difficult.

In competition with other major cotton pests *Anthonomus grandis* Boh. (Curculionidae, Coleoptera) has gained the status of key pest (Falcon & Smith, 1973 ; Rosset, personal communication). Meanwhile, the destructive impacts of pesticide based cotton growing have become clear. However, commonly Methyl Parathion or other very toxic insecticides are applied to avoid a population build-up of the Boll Weevil one month after sowing. Presumably these sprayings have strong negative effects on the available predators and parasitoids. Under integrated pest management one is therefore looking for insecticides which are less toxic to natural enemies.

One of the most common predators, particularly during the first part of the cotton growing season in Nicaragua, is *Chrysoperla externa* Hagen (Chrysopidae, Neuroptera). The available literature on the impact of pesticides on benificial cotton insects in Nicaragua is very scarce. References from the USA indicate that the related *C. carnea* is an important predator in cotton and other crops, and it seems to be less affected by insecticides than many other predators (Van Steenwijk *et al*, 1975 ; Rajakulendran & Plapp, 1982).

The toxicity tests reported here were intended to be part of a wider field- and laboratory research on effects of different (groups of) insecticides on various beneficial arthropods in cotton ; but this could only partly be carried out.

The organophosphate Methyl Parathion 48 % EC has been the "traditionally"

recommended chemical against the Boll Weevil in Nicaragua, at 0.7 (up to 1.4) l/ha, which is equivalent to 3.2 ml per l of water, or 0.15 % a.i.(Falcon & Smith, 1973, Bodan *et al*, 1979). It is a non-systemic insecticide and acaricide with contact, stomach and some respiratory action, being a cholinesterase inhibitor (Anonymus, 1990).

Cypermethrin is a relatively new, synthetic pyrethroid and in cotton it is mainly used against caterpillars (*Heliothis, Trichoplusia*), but it is also effective against Coleoptera. It is also a non-systemic, contact and stomach insecticide, commonly applied at more than 0.005 % a.i. (recommended at 20-75 g a.i./ha).

In Brasil Cypermethrin is mentioned as "the most suitable IPM tactic against *A. grandis*", applied at 3.95 g a.i./ha (Sousa Ramalho *et al*, 1990).

The IOBC/WPRS Working Group "Pesticides and Beneficial Organisms" developed standard methods for determining the side-effects of pesticides on natural enemies, including *C. carnea*, the most common lacewing in the Northern hemisphere (Hassan *et al*, 1988).

Given the conditions in Nicaragua, however, a more simple and rapid method for toxicity testing was chosen in this experiment.

The predators used for testing could not be directly taken from the field, as planned, but were reared in order to obtain larger numbers. The Boll weevils were more easily collected from cotton fields.

MATERIALS AND METHODS

Adults of *C. externa* were captured in or along cotton fields near León, Nicaragua by means of sweeping net and UV-light trap. They were kept in plastic containers (10-15 adults in each) made out of 2 l plastic bottles of which the bottom had been cut off and replaced by fine mesh, while the screw cap was perforated. The lacewings were fed with water and a solution of honey and yeast. Eggs produced on a sheet of paper along the inner surface were regularly collected for further rearing. The *C. externa* larvae were individually reared in small, 4 cm^3 cells (a method described by Sánchez & Rizo, 1987) and successively fed with (citrus) aphids, eggs of *Spodoptera* and *Trichoplusia* and with artificial diet (egg yolk, honey, water and yeast).

The Boll weevils were collected as larva or pupa in fallen cotton squares from the same region. After emergence they were fed with fresh cotton squares, honey and water. Insect rearing and testing was done in Managua.

The method for toxicity testing used here is described by Plapp & Vinson, 1977. The acetone solution of the insecticide is pipetted into a glass vial (30 ml vials in my experiment, 20 ml vials were used by Plapp). The vial is then rolled over the table to let the acetone evaporate so that a thin film of insecticide is left on the inner surface of the vial. The individual insects introduced into the vial were provided with a 1 cm piece of cotton moisted with nutrients (honey solution for adult lacewings and weevils ; the above mentioned diet for larvae of *C. externa*).

The experiments were carried out in October'90, Managua, with an average temperature of about 27 C.

In total 204 individuals of *C. externa* (84 adults and 120 larvae) and 160 adult Boll weevils were used in the experiments. With *C. externa*, adults (both sexes) and at least 3 days old were tested ; *A. grandis* was tested only as adult (both sexes) of at

least half a day old.
The insecticides used were locally bought as Cypermat 300 EC and Methyl Parathion 48 % EC. After trying standard doses (at 0, 0.01, 0.1, 1, 10 and 100 p.p.m) 5 concentrations of insecticide (resulting in between 0 and 100 % mortality) were tested, plus a control of acetone only. For both insecticides tested 1 ml of solution was applied per vial. Mortalities were counted 24, 48, 72 and 96 hours after application of the chemical.

RESULTS AND DISCUSSION

Table 1 and 2 show the results (for each replication) of the toxicity tests, as measured after 72 and 96 hours. The correct time of counting seems to be at 72 hours after application, since cumulative mortality increased during this period, but hardly thereafter.

Table 1. *Toxicity of Cypermethrin : average mortalities in % with the predator C. externa (C.ex.) and with the Boll weevil A. grandis (A.gr.) after 72 hours and 96 hours ( ). For the test method used : see text.*
(ad) = adult, (la) = larva, n = number of insects tested per concentration

Date applic.	Insect, (stage)	n	Concentration of insecticide in acetone (p.p.m.)					
			0	0.01	0.1	1	10	100
5.10	C. ex., (ad)	5	0 (0)	0 (20)	40 (40)	80 (80)	100	100
			Concentration of insecticide in acetone (p.p.m.)					
			0	0.04	0.2	1	5	25
16.10	C. ex., (ad)	4	0 (0)	0 (0)	25 (25)	0 (0)	25 (50)	75(75)
17.10	A.gr. (ad)-1	5	0 (0)	0 (0)	0 (0)	60 (80)	100	100
18.10	C.ex. (la)-1	5	0 (0)	0 (20)	0 (0)	20 (20)	0 (0)	100
18.10	A.gr. (ad)-2	8	0 (0)	0 (0)	0 (13)	0 (0)	25 (25)	88 (100)
19.10	C.ex. (la)-2	4	0 (0)	0 (0)	0 (0)	25 (25)	25 (50)	25(25)

None of the control treatments (acetone only) caused mortality, except for one case with *C. externa* larvae (17 Oct.). This exception is explained by fungus growth which developed in all concentrations of that test.
Figures 1 and 2 summarize the mortalities for each insect species for the second dose range of Cypermethrin and Methyl Parathion, whereby mortality is expressed in probits. Generally, the different LD-50 values for the two insect species tested are not far apart.
With Methyl Parathion the LD-50 for *A. grandis* lies around 2 p.p.m., that of *C. externa* larvae being only slightly lower.
With Cypermethrin, the LD-50 value for *C. externa* larvae is approx. 25 p.p.m., which is very similar to the outcome for *C. externa* adults ; however, the R-value of the latter is not significant. For the Boll weevil the LD-50 appears around 5 p.p.m.

Table 2. *Toxicity of Methyl Parathion : average mortalities in % with the predator C. externa (C.ex.) and the Boll weevil A. grandis (A.gr.) 72 hrs and 96 hrs ( ) after application. (ad) = adult, (la) = larva, n = number of insects tested per concentration*

Date applic.	Insect, (stage)	n	Concentration of insecticide in acetone (p.p.m.)					
			0	**0.01**	**0.1**	**1**	**10**	**100**
15.10	C.ex. (ad)	5	0 (0)	20 (40)	0 (20)	100	100	100
16.10	A.gr. (ad)	5	0 (0)	0 (0)	0(0)	20 (20)	100	100
17.10	C.ex. (la)	5	60*	20*	20*	40*	80*	100*
			Concentration of insecticide in acetone (p.p.m.)					
			0	**0.16**	**0.8**	**4**	**20**	**100**
19.10	C.ex. (la)	6	0 (0)	17 (33)	17 (17)	33 (33)	100	100
20.10	A.gr. (ad)	8	0 (0)	13 (13)	0 (0)	38 (38)	100	100
* this test was stopped after 72 hrs because of fungus growth								

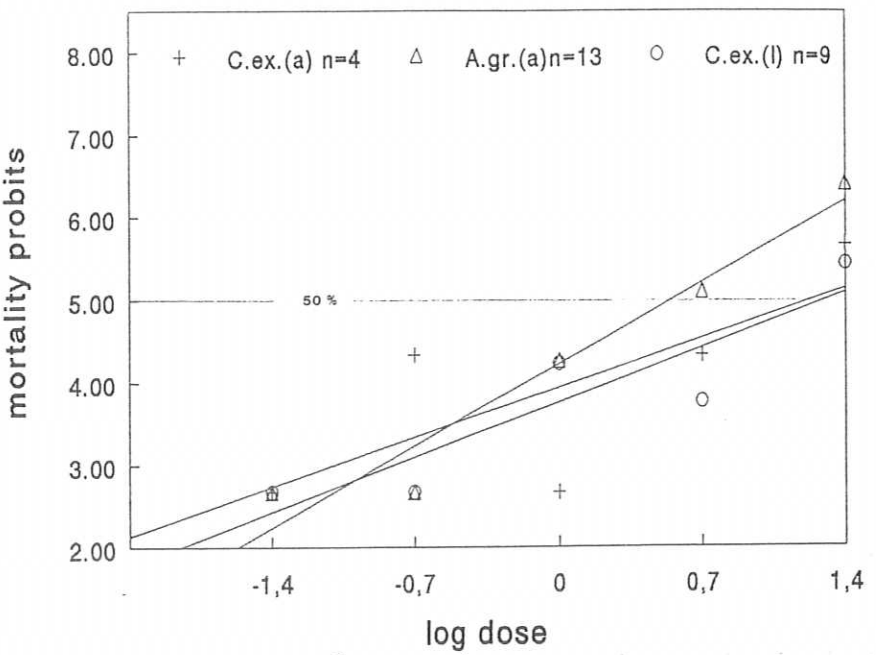

Fig. 1. *Mortality caused by Cypermethrin : log dose against mortality probits. C. externa (4 adults, 9 larvae) and A. grandis (13 adults) were exposed to 5 concentrations, apart from the control treatment. Mortality was counted after 72 hrs. See text. The lineair correlation for C. externa adults is not significant, those of A. grandis and of C. externa larvae are significant at 0.01 and 0.05 respectively. The LD-50 of the Boll weevils appears near 5 p.p.m., that of the lacewing larvae around 25 p.p.m.*

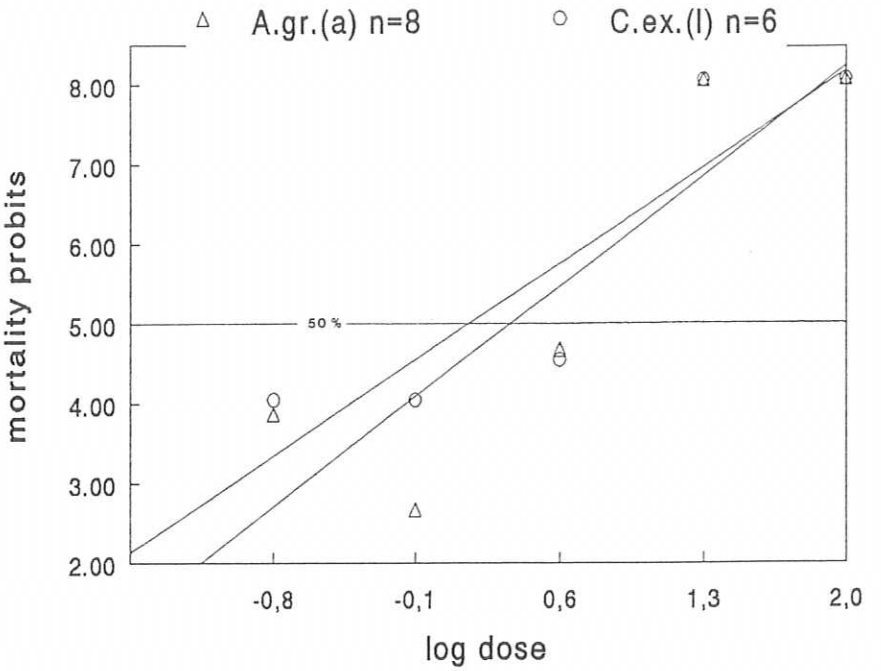

Fig. 2. Mortality caused by Methyl Parathion : log dose against mortality probits. A. grandis (8 adults) and C. externa (6 larvae) were exposed to 5 concentrations, apart from the control treatment. Mortality was counted after 72 hours. See text. Both lineair correlations are significant at 0.05 level. The LD-50 for the weevil (lower line) appears around 2 p.p.m., that of the lacewing larvae slightly lower.

A comparison between data from the USA (using the same method) and the results reported here on pesticide toxicity to *Chrysoperla* species (Table 3) show comparable values for the different tests.

The LD-50 value of Cypermethrin appears a bit lower for *C. externa* in Nicaragua, especially considering the fact that the figure of 20,3μg/vial in this experiment does not take into account the difference in vial size used (30 ml against 20 ml in USA), which undoubtedly must push the value for Nicaragua down by about 2/3, the insecticide being spread out over a larger area. The difference in toxicity could possibly also be explained by a longer or more frequent use of Cypermethrin in USA, so that the *Chrysoperla* predator could develop some resistance.

For Methyl Parathion and *Chrysoperla* larvae the LD-50 in this experiment comes out higher than that reported from the USA concerning a related species (1.1 - corrected value- against 0.26μg/vial). The higher value for *C. externa* is not very surprising, given the long history of frequent use of Methyl Parathion in Nicaragua.

Comparison of insecticide susceptibility of *C. externa* and the pest *A. grandis* shows that the former is less susceptible than the pest as far as Cypermethrin is concerned

(Fig. 1). This is in line with other reports on limited susceptibility of *Chrysoperla* species to certain synthetic pyrethroids, particularly as far as larvae are concerned (Plapp & Bull, 1978 ; Rajakulendran & Plapp, 1982 ; Hassan *et al*, 1988 ; Niemczyk *et al*, 1979 ; Kismir & Sengonca, 1980 ; Pree *et al*, 1989).
Pree *et al* (1989) even mention that *C. carnea* could be a prime candidate for mass releases of pesticide resistant beneficial organisms for use in integrated pest management programmes.

Table 3. Comparison of some data on toxicity of insecticides to Chrysoperla species (Chrysopidae). Method of Plapp & Vinson, 1977. The LD-50 is in µg/vial.

Chemical	Reference, place, year	Insect, stage (l/a)	n	Time of count (hrs)	LD-50
Cypermethrin	Rajakul.& Plapp, '82, USA	*C. carnea*, larva	20	96	22,2
	this test, ('90) Nic. *	*C. externa*, l./ad.	9/4	72, 96	20,3
Permethrin	Plapp & Bull, '78, USA	*C. carnea*, larva	12 +	72	9,87
Methyl Parathion	Plapp & Bull, '78, USA	*C. carnea*, larva	12 +	72	0,26
	this test, ('90) Nic. *	*C. externa*, larva	6	72, 96	1,6
* the vial used was of 30 ml (surface of 47 cm incl. bottom), while the other references used 20 ml vials (see method of Plapp & Vinson, 1977)					

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REFERENCES

Anonymus, 1990. The agrochemicals handbook, 2nd edition, update 5. Royal Soc. of Chemistry, Cambridge.

Bodan, R. *et al*, 1979. Manual de manejo integrado de plagas del algodonero. Comité de Control Integrado de Plagas del Algodonero. Edición especial del Banco

Nacional de Nicaragua.

Falcon, L.A. & R.F. Smith, 1973. Guidelines for integrated control of cotton insect pests. FAO, Rome.

Frisbie, R.E., 1983. Guidelines for integrated control of cotton pests. FAO Plant Production and Protection Paper no. 48.

Hassan, S.A. *et al*, 1988. Results of the fourth joint pesticide testing programme carried out by the IOBC/WPRS-Working Group "Pesticides and Beneficial Organisms". J. of Appl. Ent. 105, 321-329.

Kismir, A. & C. Sengonca, 1980. (An investigation into the effect of chemical preparations applied in the Cukurova region against cotton pests on the insect predator *Anisochrysa carnea* (Steph.)(Neuroptera, Chrysopidae). Türkiye Bitki Koruma Dergisi 4 (4), 243-250.

Niemczyk, E., M. Misczak & R. Olszak, 1979. (The toxicity of pyrethroids to predacious and parasitic insects). Roczniki Nauk Rolniczych, E, 9 (2), 105-115.

Plapp, F.W. & S.B. Vinson, 1977. Comparative toxicities of some insecticides to the tobacco budworm and its ichneumonid parasite. Envir. Entom. 6, 381-384.

Plapp, F.W. & D.L. Bull, 1978. Toxicity and selectivity of some insecticides to *Chrysopa carnea*, a predator of the Tobacco Budworm. Envir. Entom. 7 (3), 431-434.

Pree, D.J., D.E. Archibald & R.K. Morrison, 1989. Resistance to insecticides in the common green lacewing *Chrysoperla carnea* (Neuroptera : Chrysopidae) in Southern Ontario. J. of Econ. Entom. 82 (1), 29-34.

Rajakulendran, S.V. & F.W. Plapp, 1982. Comparative toxicities of five synthetic pyrethroids to the Tobacco Budworm (Lepidoptera : Noctuidae), an Ichneumonid parasite, *Campoletis sonorensis*, and a predator, *Chrysopa carnea*. J. of Econ. Entom. 75 (5), 769-772.

Sánchez, T. & R. de F. Rizo, 1987. Estudio de cría masiva y comportamiento como depredador de *Chrysoperla carnea*. Trabajo de diploma, UNAN, León, Nicaragua.

Sousa R., F. de, F.M. Marques de Jesus & J.V. Gonzaga, 1990. Tacticas de manejo integrado de pragas em áreas infestadas pelo bicudo-do-algodoeiro. Pesquisa Agropec. Bras. 25 (5), 677-690.

Steenwijk, R.A. van *et al*, 1975. Increases of *Heliothis spp.* in cotton under various insecticide treatment regimes. Envir. Entom. 4 (6), 993-996.

ATTRACTION OF MIGRATING ANTHOCORIDS BY ODOURS FROM *PSYLLA*-INFESTIONS IN A PEAR ORCHARD: THE EFFECT OF INTERRUPTING THE ODOUR SOURCE

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Key words:
Anthocoris, *Psylla*, pear psylla, infochemicals, *Orius*, population dynamics

Summary
In a field experiment the role of odours in attracting migrating anthocorid bugs was studied. Differences found between anthocorid density around induced psylla outbreaks in cages and anthocorid density around control cages indicated that the bugs were able to perceive odours emanating from the caged trees. A sudden interruption of one of the odour sources resulted in an immediate drop in bug density around the source, suggesting that the bugs were actually migrating.

INTRODUCTION

Large population growths of pear psylla, by growers often referred to as "outbreaks" are often followed by an influx of anthocorid bugs, the natural enemy of the psylla (Atger, 1982, Van der Blom *et al.*, 1985, Trapman & Blommers, 1992) These predators can quickly reduce the psylla populations to a level of only marginal economic damage for the growers (Van der Blom *et al.*, 1985). Where the anthocorids come from is not yet exactly known, but especially at large outbreaks bugs originating from outside the orchard play an important role in the suppression of the outbreak (Booij, 1990).

In two studies, it has been suggested that these allochtonous bugs use olfactory cues for the location of these large *psylla* populations. Drukker and Sabelis (1990) demonstrated by means of an Y-tube olfactometer that both *Anthocoris nemorum* and *A. nemoralis* respond to odours from from *psylla*-infested pear seedlings. In 1991 Drukker *et al.* (1992) conducted a field experiment in an orchard where three experimental trees were provided with fine meshed gauze cages. On these trees a *psylla* outbreak was induced. Around the experimental cages higher densities of anthocorids were observed than around control trees on which psylla densities were low or absent. Drukker *et al.* (1992) attributed the observed differences to arrestment or attraction of the bugs by olfactory cues.

A year later the experiment was repeated and expanded. Some results and preliminary conclusions will be presented in this paper.

MATERIAL AND METHODS

The methods were similar to those described in Drukker *et al.* (1992). On caged experimental trees psylla populations were induced, which then could be considered as

odour sources. Two sets of controls were now established: three caged trees which were sprayed with Amitraz to suppress psylla populations, and to compare natural populations of bugs and psyllas, three trees without cages were sampled. This time the experiment was initiated earlier: on May 7th in stead of June 19th. Beating samples (30) were taken for monitoring adult bug and psylla numbers around the cages. Leaf samples (30) to check the number of immature psyllas inside the cages and on adjacent trees.

At the moment the influx af anthocorids was at its peak (on the 12th of August), one of the three experimental cages was covered with an airtight plastic sheet. After two weeks the sheet was removed. In this way the effect of a suddeb and short interruption of the odour source could be studied.

RESULTS AND DISCUSSION

Except for the initial two months, the densities of *Psylla* nymphs in the experimental cages were invariably high and comparable to those of the previous year (about 5 per leaf on average). In control cages psyllas were practically absent. Natural populations on trees without cages were variable (0.6 per leaf in late June; 0.2 per leaf in mid August). Psylla

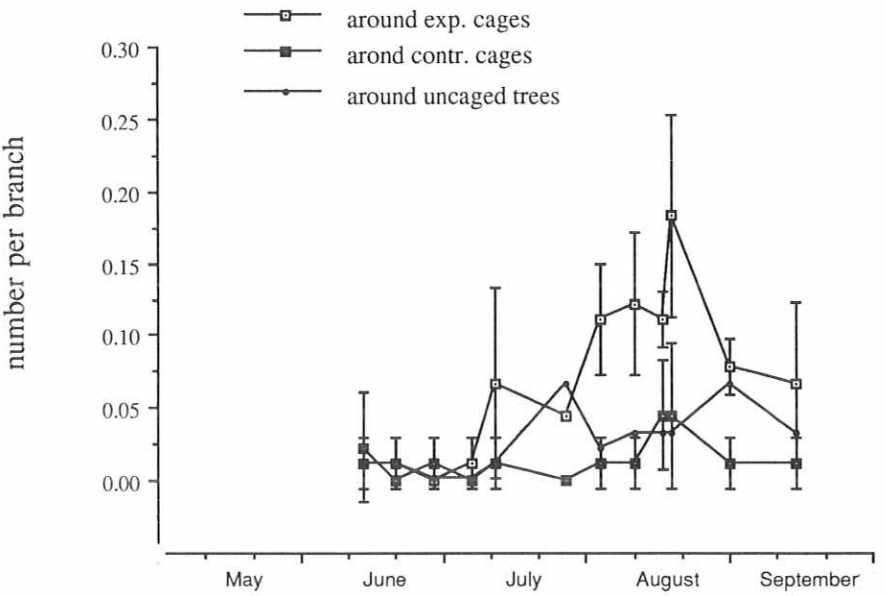

Figure 1.: Number of adult anthocorids around experimental and controltrees

nymph densities around the cages did not differ significantly from the natural densities and followed the same pattern.

Adult anthocorids were observed during the whole period (fig.1): in June and July in low numbers with no significant differences between experimental and control trees. In August a peak of adult anthocorids were found only around experimental trees, on all sample dates significantly different from both caged uninfested trees and uncaged trees with natural infestation. This finding strongly supports the hypothesis that bugs locate their prey by means of olfactory perception.

The results of the second experiment were in line with the hypothesis as well (fig.2). Adult anthocorids were observed on all of three experimental trees until the moment that one of the trees (tree 1, fig 2) was covered with air-tight plastic sheet. Two days after covering tree 1 not one bug was found, whereas the other trees continued to harbour anthocorids. After removal of the sheets bugs were again observed on tree 1. The fact that a sudden interruption of the "odour source" had an immediate effect on the observed number of bugs, is a strong indication that bugs not only react to odours, but are also influenced in their migratory activities by these odours.

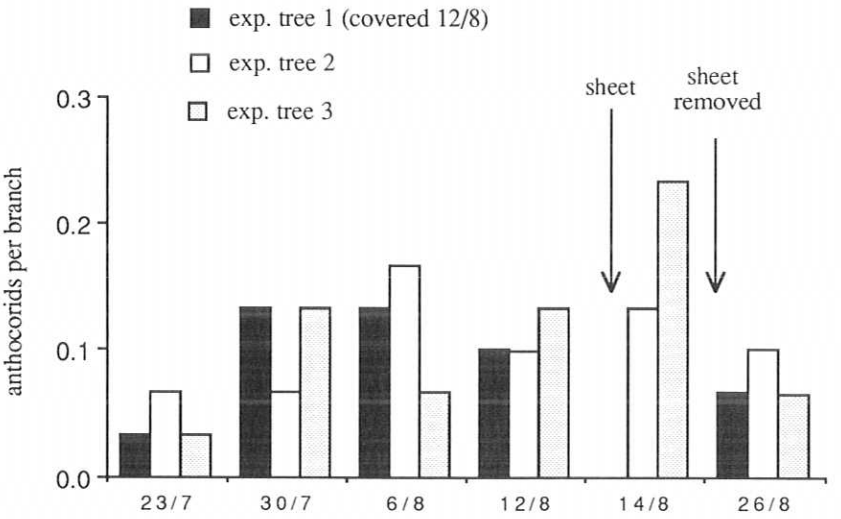

Figure 2.: Adult anthocorids around experimental trees before during and after covering one of the trees with airtight plastic sheet.

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REFERENCES

ATGER, P., 1982. Le psylle du poirier. In: C.T.I.F.L., Paris, 68pp.

BOOIJ, C. J. , 1990. A simulation model for the interaction between pear psyllids (*Psylla pyri*) and predatory bugs (*Anthocoris nemoralis*). -*Bull. IOBC/WPRS* 13(1): 55-60.

DRUKKER, B. & M. W. SABELIS, 1990. Anthocorid bugs respond to odour emanating from psylla infested pear trees. -*Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam* 1: 88-89.

DRUKKER, B., P. SCUTAREANU, L. BLOMMERS & M. W. SABELIS, 1992. Olfactory response of migrating anthocorids to psylla-infested pear trees in an orchard. -*Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam* 3: 51-56.

TRAPMAN, M. & BLOMMERS, L. 1992. An attempt to pear sucker management in the Netherlands. -*J. Appl. Entomol.* 114: 38-51.

VAN DER BLOM, J., B. DRUKKER & L. BLOMMERS, 1985. The possible significance of various groups of predators in preventing pear psylla outbreaks. -*Med. Fac. Landbouww. Rijksuniv. Gent* 50 (2a): 419-424.

KEY WORDS